

THE EFFECTS OF A VITAMIN A DEFICIENCY
ON THE REPRODUCTIVE CAPACITY OF RAMS

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INTRODUCTION

Efficient reproduction in his animals is one of the goals that any producer must achieve in order to successfully compete in today's livestock business. This underlines the importance of research in any aspect of reproductive physiology.

In the literature one finds that efforts in this area of research have been directed mostly towards studying various effects of environmental and genetic factors on female reproduction, somewhat underestimating the importance of the male; consequently the basic aspects of the male reproductive physiology have been overlooked to some extent in the past.

This study is part of a broad project being undertaken at the University of Florida to investigate the effects of various nutritional factors on the male reproductive capacity. Other studies in this project have been the work reported by Meacham (1962) and Tilton (1962) in which protein and energy have been investigated.

Lack of vitamin A is the only specific dietary deficiency that has been shown with any certainty to affect semen production in farm animals. It is also true however that the studies from which this knowledge was obtained are to some extent outdated in the light of today's improved research methods and techniques. The use of purified diets is indeed a tool of great value in animal research for it facilitates complete knowledge of the nutrients present in the diet, thus allowing a more precise characterization of the role that a specific dietary factor has in the physiology

of the organism in question. It is with this in mind that the present study was undertaken to further investigate the effects of avitaminosis A on the reproductive capacity of the ram.

Due to the ever increasing need of information related to radiation and its effects on biological systems, a study on the tolerance of vitamin A deprived animals to gamma irradiation from a Co^{60} source was included.

Also attempt was made in this study to counteract the harmful effects of vitamin A deficiency by administering Testosterone, pregnant mare serum and Thyroprotein.

LITERATURE REVIEW

Vitamin A and Reproduction in the Male

As early as 1924 it was found in experiments with rats by Gross (1924) and Wolbach and Howe (1925) that vitamin A deficiency caused pronounced atrophy of the testes. Since then extensive investigations have been carried out to elucidate the influence of this deficiency on the male germinal epithelium in laboratory and farm animals. In addition to atrophy of the testes this deficiency will also cause impaired development of the epididymis, seminal vesicles, and prostate in young rats. Injection of testosterone can obviate this atrophy in vitamin A deficient rats as shown by Mayer and Truant (1949). Further injections of gonadotrophic hormone will stimulate the development of the accessory sex glands in vitamin A deficient rats according to Mayer and Goddard (1951). This suggests that this deficiency causes a lowered excretion of pituitary gonadotrophic hormone or a lowered response to this hormone by the interstitial cells of the testes.

Recent work by Howell et al. (1963) showed that male rats maintained on a diet in which the vitamin A alcohol had been replaced by vitamin A acid developed lesions in the reproductive tract. The testicular changes were a sloughing of the cells of the germinal epithelium followed by an obliteration of the lumen of the tubule by sertoli cells. Testicular regeneration was produced by the administration of vitamin A alcohol. The lesions were comparable to those of vitamin A deficiency as described by

Mason (1933) but were uncomplicated by the secondary manifestations of vitamin A deficiency. This paper is the first to describe lesions in the testes of healthy growing prepubertal rats fed the vitamin A acid diet. It is widely accepted that vitamin A acid can perform all the functions of vitamin A alcohol except for its part in vision. Dowling and Wald (1960) summarized the role of vitamin A acid in a recent symposia on vitamin A. The general tissue functions of vitamin A that support growth and maintenance in the rat are served also by vitamin A acid; but since the rat does not reduce this substance, it forms neither the alcohol, the form in which vitamin A is stored, nor the aldehyde (retinene) needed for the synthesis of visual pigments. For this reason, rats maintained on vitamin A acid, though growing normally and otherwise in good condition, become extremely night blind and eventually blind. The failure to form visual pigments also has specific anatomical consequences; the outer segments of the visual cells deteriorate, followed by the loss of all but a remnant of the cells themselves, in an otherwise normal retina. The findings of Howell et al. (1963) do not support the statements of Dowling and Wald (1960).

In bulls a pronounced vitamin A deficiency delays sexual maturity, suppresses sexual interests and causes testicular degeneration and thus a lowering of sperm production and quality. This has been shown by Bratton et al. (1948), Erb et al. (1947), and Guilbert and Hart (1935). Bratton et al. (1948) studied the effect of rations low in carotene on the breeding behavior, spermatogenesis and semen production of mature dairy bulls. Levels of 2 to 3 mcg. of vitamin A per 100 ml. of blood were recorded before any of the deficiency symptoms appeared.

Incoordination and loss of the ability to mount without loss of libido were the first signs to appear and they occurred before any marked impairment on semen quality was evident. Percentage of motile spermatozoa was decreased. The per cent of abnormal spermatozoa was increased. Histological examination of the testes showed degeneration of the germinal epithelium of the seminiferous tubules. Hogdson et al. (1946) also found that vitamin A deficiency apparently does not cause severe disturbance in spermatogenesis until the animals are in advanced stages of deficiency.

Erb et al. (1947), Hogdson et al. (1946), Jones et al. (1946) and Madsen et al. (1942) have reported that pituitaries are often cystic in the vitamin A deficient bovine. These findings suggested the possibility that vitamin A deficiency may exert its deleterious effect on reproduction indirectly through the anterior pituitary gland. Gunn et al. (1942) improved the semen quality of a ram deficient in vitamin A by injections of pregnant mare serum. Hogdson et al. (1946) obtained similar results with a vitamin A deficient bull. However Lindley et al. (1949) reported that testosterone propionate injected semiweekly in dosages of 12.5 mg. before and during the deficient condition or of 37.5 mg. after vitamin A deficiency symptoms began to appear resulted in no improvement on semen quality of vitamin A deficient rams. Similar results were obtained from injections of pregnant mare serum. This work suggests differences between rat and sheep since Mayer and Truant (1949) were successful in preventing testicular atrophy in the rat resulting from a vitamin A deficiency by administration of testosterone propionate.

Pathology of Vitamin A Deficiency

Moore (1960) described at least three basic lesions occurring in avitaminosis A: (1) Lack of vitamin A, required in the form of its aldehyde for the formation of rhodopsin, causes defective dark adaptation unless complications have ensued this lesion may be regarded as "biochemical" rather than "structural." Thus the response to treatment with vitamin A is rapid and dramatic. (2) Lack of vitamin A causes xerosis or keratinization of membranes in many parts of the body, the well-known xerophthalmia belongs to this group of lesions. Epithelia tends to become dry and excessively thick and horny. Those membranes having a columnar structure, often associated with secretions of mucus give place to thick layers of stratified epithelia as found in the outerpart of the epidermis. The most common secondary effects of avitaminosis A arise from the bacterial infection of the abnormal membranes. In some sites such as the urinary bladder, the injury to the membrane may sometimes induce calcification with the formation of stones. (3) During growth, lack of vitamin A can cause defective modeling of the bones. As a result the bones are not compact, strong and well shaped, but cancellous, weak and excessively thick.

Lesions of more than a single type are often super-imposed, and the possibility of further types cannot always be regarded as secondary effects of the bone lesions. Another abnormality is an increase in the pressure of spinal fluid sometimes associated with hydrocephalus. These lesions might well be regarded as secondary effects of malformation of the skull bones, but a rival theory suggests that deficiency of the vitamin causes the choroid plexus to secrete unduly large amounts of fluid.

Vitamin A (general)

Vitamin A is an alcohol and forms esters with fatty acids. Most of the vitamin A in the livers of both mammals and fish is present in the sterified form (White et al. 1959 and Harper 1963). Conversion of provitamin A (carotene) takes place in the intestinal wall in rats, pigs, goats, rabbits, chickens and sheep. According to Barnett and Reid (1961) carotene may be found in the liver of animals suffering from a vitamin A deficiency and does not appear to be capable of utilization for vitamin A synthesis. Dowling (1960) summarized today's views on vitamin A metabolism as follows: Vitamin A alcohol, the transport form of the vitamin and the storage form as the ester, is oxidized to retinene because of the rapid removal of the aldehyde in its combination with opsin to form rhodopsin. The alcohol is oxidized to the acid in the liver and it is rapidly used in its function of growth and tissue maintenance. Acid is the important form for all functions other than visual, it must be stored as the alcohol and cannot be reconverted to either aldehyde or alcohol.

Most of the information on the concentration of vitamin A in the various organs has been gained in studies on rats. Moore (1931) used the antimony trichloride reaction, with the aid of a Lovibond Tintometer, in studies of vitamin A in the tissues of rats which had been given liberal amounts of carotene. By this treatment total stores of up to 50,000 I.U. of vitamin A were accumulated in the liver. Apart from the intestine which contained much yellow pigment the rest of the carcass contributed 100 I.U. of vitamin A. When the various tissues were examined separately the intraperitoneal fat contained 0.5, 5 and 5 I.U. in four experiments, and the kidneys and lungs each 0.0 and 5 I.U. in three experiments.

No vitamin A could be detected in the brain, heart, pancreas, spleen, thymus or testes. The method of analysis, however, was too crude. Davies and Moore (1934) estimated vitamin A in the organs of rats which had been given either carotene or the preformed vitamin. Rats which had been dosed with carotene until their livers contained 30-90 I.U. vitamin A per gram had 1-3 I.U. of vitamin A per gram in kidney. Similar amounts were found in the lungs of two out of five animals but not in the remaining three animals. By giving vitamin A in large non-toxic doses concentrations of 25,000 I.U. per gram liver, 30 I.U. per gram kidney and 250 I.U. per gram of lungs were obtained. The intraperitoneal fat deposits had 50 I.U. per gram and the adrenal glands 1500 I.U. per gram. By raising the dose in the toxic region the liver contained up to 36,000 I.U. per gram, the kidneys 360 I.U., lungs 600 I.U. and suprarenals 30 I.U. Concentrations of up to 15 I.U. per gram were found in the muscles, heart, spleen and brain.

The total storage of carotene and vitamin A in the liver and depot fat of cows which had access to a carotene rich rations throughout life was estimated to be 0.6 to 0.7 gram of vitamin A for the younger animals and up to 3.6 grams in aged cows (Maynard and Loosli 1962). From 67 to 93 per cent of the storage was in the liver. In this organ most of the storage was in the form of the vitamin itself but in the fat depots carotene predominated. This work reported was done by Gullbert and Hart (1934 and 1935). Eaton (1951) has shown that in dairy calves approximately four months of age fed a depletion ration devoid of vitamin A activity, the plasma vitamin A level decreases in a linear manner until values of 4 mcg. per 100 ml. are reached. Calves exhibiting

such low plasma values for two consecutive weeks were shown to be depleted of liver stores of vitamin A. Unpublished data by Dr. R. L. Shirley and co-workers showed that cattle receiving 30,000 I.U. per day and those receiving no supplemental vitamin A had blood plasma levels of 54 and 34 respectively. Another trial conducted during the summer showed levels of 46 and 18 mcg. of vitamin A per 100 ml. of plasma for groups receiving the supplemental vitamin A and no vitamin A respectively. Vitamin A in liver expressed as mcg. per gram of liver dry weight was found to be 119 and 78 for the supplemented group during the first and second trial respectively and 17 and 7 for the second group during the first and second trial respectively. In the second trial vitamin A level in the heart was found to be 0.17 and .05 mcg. per gram of heart for the supplemented and non-supplemented groups respectively.

In sheep vitamin A storage has not been as extensively studied as it has been in rats. Harm (1942) reported the average vitamin A content in a gram of liver as 503 I.U. in 19 animals. Moore and Payne (1942) reported levels of 460 I.U. per gram of liver in 20 animals. The above data were expressed in terms of grams of dry liver. Recent data in the literature indicate the levels of vitamin A in the liver of sheep to be within a range from 25 to 54 mcg. per gram of fresh liver, Cline (1962), Goodrich (1962) and Cline et al. (1963). Data on the vitamin A content of tissues other than blood and liver are lacking for sheep.

The Thyroid and its Role in Reproduction and Vitamin A Metabolism

The role of thyroxine in reproduction has been most extensively studied among the hormones that may directly or indirectly influence the

secretion rate of hormones influencing reproductive activity such as the gonadotrophs and the hormones of the ovary and testes.

Berliner and Warbritton (1937) reported that thyroidectomy of rams produced a decrease in semen volume, spermatozoan concentration and an increase in the relative numbers of abnormal sperm. Thyroxine administered to the thyroidectomized animal resulted in the production of semen with an increased number of sperm and decreased percentage of abnormal spermatozoa. Bogart and Mayer (1946) reported that treatment with thyroxine and thyroprotein alleviated symptoms of "summer sterility" resulting from impaired spermatogenic activity. The feeding of thiouracil during the fall breeding season maintained semen characteristics typical of the summer months. It was concluded in this study that the thyroid gland is of major importance in the reproductive physiology of the ram. Bhatnagar (1955) studied the seasonal variation in the histology of the thyroid and the testes of Indian buffalo bulls. They found considerable seasonal variations in the histological structures of the two glands, they also observed that during the period when the quality of semen deteriorates in buffalo the secretory activities of the two glands as shown by their histological studies appears to decrease. Goswami (1962) conducted an experiment to study the effect of thyroxine and pregnant mare serum (PMS) hormones on reaction time and semen quality of buffalo bulls. The experiment was conducted in India from June to February. One thousand I.U. of PMS were administered by weekly subcutaneous injections. A daily dosage of 100 mg. of thyroxine was administered orally. Treatment with thyroxine and PMS did not influence the reaction time of the animals.

Semen volume was reduced significantly in the groups receiving thyroxine and PMS. Initial motility was improved in both groups during treatment. No effect from either hormone was noticed on sperm concentration or total number of spermatozoa during the treatment. The percentage of abnormal spermatozoa was decreased with thyroxine treatment. PMS treatment initially resulted in an increased percentage of abnormal cells but prolonged administration checked this increase.

Black et al. (1950) studied the effect of testosterone propionate and thyroprotein in rams. Ten mg. of testosterone and 1 gm. thyroprotein were administered daily by subcutaneous injections and orally respectively. One gm. of thyroprotein per hundred lbs. of body weight was given orally to a second group. A third group received both testosterone and thyroprotein at the same level administered to groups one and two. Thyroprotein reduced weight, decreased libido and had no effect on sperm motility, volume, concentration or number of ejaculates. The testosterone treated group showed a significant decrease in semen concentration. They also reported that testosterone could prevent the loss of libido caused by treatment with thyroprotein.

Recent studies by Brooks (1961), do not support the observations of Berliner and Warbritton (1937), and Bogart (1946). Brooks (1961) injected thyroxine at levels of 2, 3, or 4 mg. per hundred lbs. of body weight to rams. Considering the fact that 1 mg. of thyroxine injected equals approximately 1 gm. of thyroprotein fed (Cole and Cupps 1959) the dosages used in the experiments of Brooks (1961) are comparable to dosages used in previous work in this field. The results obtained by Brooks (1961) indicated that injections of

thyroxine at high temperatures accentuated the detrimental effect on semen quality or libido. Tapazole was also tested and its use produced a hypothyroid condition but this had no effect on semen. However one must be cautious in making any generalization at this point since the work so far reported was done under a wide range of environmental conditions which would undoubtedly have some effect on the thyroid needs of these animals. Such authors as Turner (1961), Cole and Cupp (1959) and Hafez (1962) agree that the thyroid gland is involved in the reproductive process but in a poorly understood fashion.

Another poorly understood function of the thyroid gland is its relationship to vitamin A metabolism. Drill (1943) reviewed this subject and reached several conclusions: (1) The lack of vitamin A increased thyroid activity and hypervitaminosis had the opposite effect. (2) Deficiency in vitamin A will produce hypertrophy of the thyroid. (3) Experimental hyperthyroidism increases requirements for vitamin A. (4) In the absence of thyroid carotene could not be converted to vitamin A. Some of these observations have been confirmed by work done since Drill published his review in 1943. Johnson and Bauman (1947) found that thyroxine increases expenditures of vitamin A deficient rats. However they point out that their results were complicated by accelerated growth observed in the thyroxine fed rats, since this would mean an increase in the animal needs for nutrients including vitamin A. Reddy and Thomas (1962) working with calves, rabbits and bulls reported that in hypothyroidism conversion of carotene to vitamin A was reduced but unaltered in hyperthyroidism. Their experimental numbers of animals were limited.

Cline et al. (1963) found that feeding 150 mg. of tapazole per day or 100 mcg. of triiodo-l-thyronine did not affect liver storages of vitamin A. Any conclusion drawn from work where histological examination of the thyroid glands were not made to confirm thyroidal status should be made with caution.

Moore (1957) in his book of vitamin A reviewed the interrelationships of thyroid and vitamin A metabolism. He concluded at the time that the usual effect of thyroxine will be to accelerate any prevailing trend in vitamin A metabolism. If conditions are conducive to expenditures the rate of expenditure is increased. If dietary conditions are conducive to the storage of vitamin A the amounts stored will be increased by thyroxine, however the why and how of this remains unsolved today.

Histology

Warbritton and McKenzie (1937) described the pituitary glands of ewes in various phases of reproduction. They found the pituitary body of the ewe to be very complex. They were unable to classify the cells of the pituitary into the conventional acidophils, and chromophobes. They found 9 morphologically different types of cells which they didn't think necessarily represented distinct functional types. In this study the different regions of the pituitary were found to be conspicuously different, that counts made at one level would not be valid for another or for the whole gland. At the time of the studies by Warbritton and McKenzie (1937) present refined histological techniques were not available.

Based on the chemical nature of the secretory products of the various pituitary cells Pearse (1950) and Wilson and Ezrin (1954) have proposed differential staining of the anterior hypophysis. The periodic acid Schiff technique (PAS) of Pearse (1950) was used by Jubb and McEntee (1955) in the study of the bovine pituitary. They were able to classify granule containing cells stainable by PAS into Beta and Delta types on the basis of positional morphological and functional differences. Beta cells were described as large polyangular finely granulated, and with an ill-defined cytoplasmic outline. They were concentrated in the medulla and sparsely scattered in the cortex of the anterior lobe. The functional relationship of the Beta cells identify them with thyrotropic hormone production. These cells were enlarged, degranulated and vacuolated in thyroid deficiency. The Delta cells were much smaller, rounded and sharply outlined with coarser granules which were irregular in size and shape. These cells were present in all parts of the adenohypophysis but concentrated in the medulla of the anterior lobe. These cells were identifiable with gonadotrophic activity especially LH. They were unable to divide the acidophils. Purves and Griesbach (1954, 1955) described two categories of gonadotrophs or delta cells in the rat using the PAS method. One type they found restricted to peripheral regions and the other to central areas of the glands. In addition to finding those differences in distribution, they have stated that the peripheral gonadotrophs have a coarser granulation and give a more intense reaction with PAS. On the basis of changes occurring in these two types of gonadotrophs after testosterone treatment and after varying periods of gonadectomy these workers suggested that

their "peripheral" gonadotrophs produced FSH and their "central" gonadotrophs produced LH.

Wilson and Ezrin (1954) described a modified PAS method in which the Schiff reaction is followed by a methyl blue counterstain by which some but not all of the PAS reactive cells changed from red color to purple color. These workers concluded that the "PAS-red" cell represented the Beta cell or thyrotroph and the "PAS-purple" cell the Delta cell or gonadotroph. Rennels (1957) used a modification of the PAS-methyl blue method which in conjunction with aldehyde fuchsin, enables the tinctorial differentiation of two types of gonadotrophs in the pituitary gland of the rat. These two cell types were both gonadotrophic in function as indicated by their development into distinct PAS-red and PAS-purple castration cells following gonadectomy. Thyroidectomy cells were found to be PAS-negative, aldehyde fuchsin-negative and to have an affinity for orange G. Sanders and Rennels (1959) found the predominant chromophilic cell type in grafts producing luteotrophin (LTH) to be an elongated acidophil staining selectively with orange G when the azan stain is used. Pearse (1960), Humason (1962) and Ham and Leeson (1961) were also used as references for proper histological techniques, in this dissertation.

Effects of Irradiation on Reproduction

Schubert and Lapp (1958) described the spermatogonia as the most radiosensitive cell in the human body and indicated that 50 roentgens inhibits their development, permanent sterility for the human male requires about 500 to 600 roentgens while 250 roentgens may produce sterility for one to two years. Seminal fluid production was not affected

but no living sperm were present. The germ cell damage proved dose dependent and proportional to dose down to the lowest levels investigated. Craig et al. (1961) studied the effect of whole-body irradiation on the fertility of the male rat and mouse by systematic weekly matings of the treated animals. In both species irradiation with 300 roentgens produces a short period of sterility associated with oligospermia. This occurred about 45 days after treatment in the mouse and 65 days in the rat. Histological examination showed that the main effect is on the spermatogonial stages but there are obvious differences in the timing and extent of these irradiation effects. The time from treatment to the onset of sterility is a measure of the overall duration of spermatogenesis. The highest dose level used 500 roentgens did not cause a rapid onset of sterility in contrast to the action of some alkylating agents, which rapidly induce infertility. In the cock X-ray doses of up to 10,000 roentgens has been shown by Kosin (1944) to have no detectable effect on semen motility but the fertilizing capacity of spermatozoa was markedly reduced after the exposure to 200 roentgens, and was destroyed altogether after a dose of 500, supposedly due to damaged chromatin. Mann (1954) found that rams exposed to 100,000 roentgens and examined immediately after exposure had normal motile spermatozoa in their semen, fructolysis and adenosine triphosphate content were also normal. This would indicate that if any damage was done to the ram semen it must be ascertained through other criteria. Murphree and Parish (1956) reported that when mature beef bulls were exposed to a single dose of 100-400 roentgens whole-body gamma irradiation, the first observed semen changes were an increase in percent abnormal sperm at six weeks after irradiation and a decrease in sperm

concentration at eight weeks after irradiation. Freund and Murphree (1960) found that whole body gamma irradiation of mature Hereford bulls with a single dose of 400 roentgens from a cobalt 60 source did not result in any apparent change in semen characteristics (sperm concentration and initial fructose level) or in sperm activity (initial motility and fructolytic activity) during a three weeks post-irradiation period. Pace et al. (1962) showed that fructose utilization per 100 sperm of bulls was significantly depressed during the 8th, 11th, and 12th weeks following whole body gamma irradiation with 400 roentgens.

The effects of irradiation on semen characteristics of animals under nutritional stress of any kind were not available.

Effects of Irradiation Compounded with Vitamin A Deficiency

The only work found in which irradiation was involved with vitamin A deficiency was done by Meacham et al. (1962) working with swine. Sixty crossbred swine were allotted to two groups of 30 each. One received a basal ration containing only traces of vitamin A, the other group received the same ration supplemented with 2,000 I.U. of vitamin A acetate per pound of feed. After 13 weeks on the ration, 10 animals of each lot were chosen at random for irradiation. These pigs were subjected to 350 roentgens of whole-body gamma irradiation from a 6,000 curie cobalt 60 facility. By the end of 15 days, all of the control and nine of the 10 vitamin A deprived animals had died. The average survival periods for control pigs and those fed the low vitamin A diet were 234 and 286 hours respectively. Other than this no variation in irradiation response could be attributed to vitamin A intake of the pigs. The major symptoms of irradiation damage

were: depression, skin hemorrhaging from eyes and mouth, increased respiration, mild muscular spasms just prior to death, extensive hemorrhage of lymph nodes plus varying amounts of hemorrhage in the kidneys, heart, urinary bladder, intestine and stomach. Shirley et al. (1962) in conjunction with Meacham on the above experiment found that vitamin A supplementation decreased the deposition of copper in the liver while copper supplementation increased the level of vitamin A in the liver. Gamma irradiation was shown to increase the deposition of copper in the liver, but had no significant effect on the deposition of vitamin A in the tissue.

EXPERIMENTAL PROCEDURE

Materials

Animals

The animals used in this study were 48, 20-months old Florida native rams from Mr. L. A. Maxie's ranch in Frostproof, Florida. These rams were brought as yearlings to the University In June 1962. At this time they were placed in a preliminary experiment in which 12 received 5,000 I.U. of vitamin A per head per day. The remaining 36 received no supplemental vitamin A. The rams were fed rations consisting of 25 per cent cottonseed meal and 75 per cent white corn meal. All rams had access to coastal bermuda hay at all times. This preliminary experiment was continued until January, 1963. At this time two rams were brought in as replacements, from the University Sheep Unit for two that died. From January to February the rams were given a period of adjustment to a new purified diet to be used in the study here reported. On February 2, 1963, the experiment described henceforth was initiated. The average weight of the animals at the start was 87 lbs. with a range from 115 to 74 lbs. The twelve rams that had previously been receiving vitamin A were put on the control diet consisting of a purified ration shown in Table 2 and 3, containing 3,000 I.U. of vitamin A per lb. of feed. The remaining 36 rams were placed on the vitamin A free diet.

Assignment to treatments was accomplished in the following manner. Vitamin A deprived animals were not assigned to a treatment group until

blood analysis showed 15 mcg. or less of vitamin A per 100 ml. of plasma. A liver biopsy was also performed when such levels were reached. When 12 animals were accumulated which met the above criteria they were then assigned at random, two to each of 6 treatments. Treatments consisted of an irradiated group (A^- Irradiated), a second group received 60 R.U. of PMS/100 lbs. body weight administered daily by subcutaneous injections (A^- + PMS). A third group received daily intramuscular injections of testosterone propionate at a rate of 10 mg./100 lbs. body weight (A^- + Testosterone). A fourth group was given daily capsules of thyroprotein, 1 gram/100 lbs. body weight (A^- + Thyroprotein). A fifth group was placed on the vitamin A containing diet (returned) and the sixth group was continued on the deficient diet without further treatment (deficient).

At the same time rams that had been receiving vitamin A were also randomly divided, two to each group, a control (control) and a group exposed to irradiation (control irradiated). In this manner eight treatment groups were formed. This procedure was repeated three times so as to allot the experimental animals in their totality. The experimental procedure is outlined in Table 1. One of the animals assigned to the deficient group died in the biopsy operation and was excluded from the experimental data.

The experiment was conducted at the Physiology Barn at the University of Florida. The area of the barn used in this experiment contained 8 pens, 12 feet wide and 48 feet long, and all pens had concrete floors. A metal roof covered the feed area and 12 feet of the pen. The pens were adjacently placed and one water tank served two pens.

TABLE I
EXPERIMENTAL DESIGN

		48		36	
12				Vitamin A ⁻	
control	irradiated	deficient	A ⁻ irradiated ^b	returned ^c	A ⁻ + PMS ^d
2	2	1 ^a	2	2	2
2	2	2	2	2	2
2	2	2	2	2	2
		A ⁻ irradiated ^b		A ⁻ + Testosterone ^e	
				A ⁻ + Thyroid protein ^f	

^aThe other member of the pair died in biopsy operation.

^bThese animals were exposed to 400r at 3.27 r/min. from Co⁶⁰ source.

^cThese animals were returned to a normal vitamin A containing diet.

^dThese animals received PMS 60 R.U./100 lbs. body weight/head/day by subcutaneous injections.

^eThese animals received Testosterone propionate 10 mg./100 lbs. body weight/head/day by intramuscular injections.

^fThese animals received 1 gm. of thyroprotein/100 lbs. of body weight/head/day orally.

Scales and a working area were available in which to handle the rams. A small portable chute was used to restrain the rams during the semen collection with the electroejaculator.

Rations

The composition of the experimental rations is recorded in Table 2. Solka-floc was used to supply fiber in the rations. Corn oil was included in the ration to supply fat, to improve palatability and to insure a uniform blending of the fine texture ingredients with the coarser materials in the rations. Casein was the protein source and corn sugar and starch were included for energy.

The mineral, mineral premix and vitamin mixtures used in the rations are shown in Table 3. The mixtures containing 13 essential mineral elements was essentially the same as reported by Meacham (1962) and Tilton (1962).

Vitamins A, D, E and choline, in a corn sugar carrier were added to each ration except that the vitamin A was deleted from that of the vitamin A deprived sheep.

Methods

Management and Feeding

The rams were fed daily at 8:00 a.m. all the feed they would clean up by the next feeding. Feed refusal was weighed back daily. Fresh water was available at all times. The rams were maintained in the pens at all times except when removed to collect experimental data and for daily hormone treatment. There was one pen for each treatment group and six rams

TABLE 2
COMPOSITION OF EXPERIMENTAL RATION

Ingredient	Per cent in Ration
Cellulose (Solka-floc)	20
Casein (90 per cent protein)	20
Corn Starch	23
Corn Sugar	25
Corn Oil	4
Trace Mineral Premix	0.5
Vitamin Pre-mix	1.0 ^a
Minerals	6.5
	100.00

^aControl group received 3,000 I.U. of vitamin A per lb. of feed.

TABLE 3

COMPOSITION OF THE MINERALS, MINERAL PREMIX, AND VITAMIN MIXTURES USED IN EXPERIMENTAL RATIONS

A. <u>Minerals</u>	<u>Gm./100 lbs. Feed</u>	<u>Lbs./100 lbs. Feed</u>
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	1,444.2	3.18
$\text{K}_2\text{CO}_3 \cdot 1\frac{1}{2}\text{H}_2\text{O}$	930.7	2.05
$\text{MgSO}_4 \cdot 3\text{H}_2\text{O}$	316.9	0.70
NaCl	218.8	0.50
B. <u>Mineral Premix</u> ^a	<u>Gm./100 lbs. Feed</u>	<u>Gm./Ton Feed</u>
$\text{FeSO}_4 \cdot 2\text{H}_2\text{O}$	39.8	796.0
$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$	5.6	112.0
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	2.9	58.0
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	8.0	160.0
KI	0.88	17.6
CuSO_4	1.18	23.6
$2\text{CoCO}_3 \cdot \text{Co}(\text{OH})_2$	0.016	0.32
MoO_3	0.023	0.460
Corn Sugar (Carrier)		1,167.98 gm. or 2.57 lbs.
		7.43
		10.00 lbs.
C. <u>Vitamin Premix</u> ^b	<u>Gm./Ton Feed</u>	<u>Units/lb. Feed</u>
Vitamin A (250,000 I.U./gm.) ^c	24.0	3,000 I.U.
Vitamin D (3,000 I.U./gm.)	200.0	300 I.U.
Vitamin E (100,000 I.U./lb.)	160.0	20 mg.
Choline Chloride (25 per cent Choline)	4,000.0	500 mg.
TOTAL	4,384.0	9.65 lbs.
Corn Sugar (Carrier)		10.35
		20.00 lbs.

^aOne-half pound mixed with each 100 pounds feed.

^bOne pound mixed with each 100 pounds feed.

^cAdd 24 gm. of corn sugar to the vitamin A deprived ration.

were assigned to each pen. The rams were weighed every two weeks during the experimental period.

The hormones were administered daily and all other animals received a daily injection of physiological saline solution to have equal handling.

Irradiation

Irradiation of the rams was performed at the University of Florida Co⁶⁰ facility. Rams were irradiated at the time all the other treatments were started. The animals were restrained in wooden crates and subjected to whole body gamma irradiation at the rate of 3.27 roentgens per minute until a total dose of 400 roentgens had been administered. Two hundred roentgens were administered to each half of the body.

Semen Evaluation

Semen was collected using an electroejaculator. A monthly collection was made on all animals prior to allotment to the eight treatments. A week prior to assignment to a treatment semen was collected eight times, four times in each of two consecutive days. During the first week following initiation of the various treatments, semen was collected four times in one day. For the remainder of the experiment semen was collected once a week.

Volume of semen was determined directly by collecting the semen in graduated centrifuge tubes. Motility was evaluated to the nearest 5 per cent by microscopic examination (X430) of the cells with progressive forward movement. A blood hemocytometer was used to determine the number of sperm per cubic millimeter. From assignment of the various groups to

their respective treatments until slaughter a period of twenty-one weeks elapsed, during this time semen collected was evaluated for additional data on percentage of abnormal cells present at each collection.

Blood Evaluation

Blood samples were taken initially and at intervals of 28 days until the biopsy was performed. Blood was evaluated for vitamin A according to the method of Kimble (1939) based on the Carr-Price reaction.

Liver Biopsy

In order to have a more reliable knowledge of the true reserves of vitamin A in the liver of the animals to be assigned to the various treatments a liver biopsy was performed prior to reallotment. This biopsy was done by Dr. Fred Neal from the Veterinary Science Department of the University of Florida.

Essentially the technique consisted of the following. A midlateral incision was made in the area immediately behind the last rib on the right side of the body. A piece of liver was removed by hand of sufficient size as to allow vitamin A determinations on it. The incision was then closed and 10 cc. of penicillin were injected to prevent further infection.

Slaughter Data

On the twenty-first week from assignment to treatment groups, those rams that had survived were slaughtered. Of the 15 rams allotted in the first group during the last week of April, 10 survived and were slaughtered in the second week of September.

Of the second group 4 rams survived. This group had been allotted in the third week of June and was terminated in the second week of November. The third group had been allotted in the second week of July and was slaughtered in the first week of December. Of this last group 6 survived until termination of the experiment.

Weights of the pituitary, thyroid, adrenal, seminal vesicle, cowpers glands, testes and epididymis were taken.

At slaughter vitamin A values for blood were determined.

Those animals that died before termination of the experiment were examined for gross pathological symptoms as to cause of death. The testes, thyroid and pituitary gland were saved for histological studies from those rams that were seen soon after death.

Histological Investigation

Tissue samples from the testes, thyroid and pituitary were taken and fixed in 10 per cent formalin solution for histological studies. Thyroid tissue was taken only from animals on the control, thyroprotein and recovery treatment. The procedures of Humason (1962) were followed for fixing, inbedding, slicing and staining with eosin-hematoxylin stain. The procedure outlined by Wilson and Ezrin (1954) was followed to study the different cell types present in the adenohypophysis.

Microscopic evaluations were made (with a calibrated eye piece) of the size and the development of the secretory tissues in the testes and thyroid. The different cell types in the adenohypophysis were studied for evidence of cytoplasmic degranulation and vacuolation.

Statistical Analysis

The daily gain data were summarized into a Phase I and Phase II and Phases I and II were combined to facilitate statistical analysis. Phase I extended from the date the animals started on the purified diet to the time they were assigned to the various treatments. Phase II extended from the end of Phase I to termination of the experiment after 20 weeks.

Data on daily gains, survival time and gland size were analyzed by the analysis of variance method. Semen data was analyzed for weekly differences in semen volume, per cent motile sperm cells, abnormal sperm cells and total sperm cells per ejaculate. Only the first ten weeks of Phase II were analyzed since the reduced numbers present in each group beyond the 10th week did not merit a statistical analysis.* Semen data analysis was programmed by Dr. A. E. Brandt from the University of Florida Statistics Department, and was processed by the University's Computing Center personnel.

When a significant effect due to treatment was found Duncan's Multiple Range Test was used to seek individual treatments effect on daily gain, survival time and gland weights. Differences in semen characteristics between treatments were determined by the matrix vector product method.

RESULTS AND DISCUSSION

General Symptoms of Vitamin A Deficiency

Approximately two and a half months after assignment to the purified diet night blindness was observed in some of the experimental animals on the vitamin A deficient diet. The rams were checked for night blindness by placing a panel across an alley after dark and checking the rams individually for their ability to avoid it when driven through the alley. All animals on the deficient diet eventually showed symptoms of night blindness.

There was considerable variation in time of onset and severity of symptoms. Some rams showed a characteristic cocking of the head to one side similar to that described by Lindley et al. (1949). A number of rams showed a characteristic cloudy cornea. In some, this cloudiness **disappeared** but in most it persisted. Figure 1 illustrates this cornea condition in one of the vitamin A deficient rams. Most rams on the deficient diet eventually exhibited an uncoordinated gait and progressive weakness which became so severe in some cases that they were unable to stand as shown in Figure 2. Several rams walked on their knees, apparently as a result of loss of control over the lower part of the leg. This condition was observed in only 3 of the 35 vitamin A deficient rams. Other symptoms exhibited by the deficient rams included loss of appetite and evidence of pain.

It is interesting to note that some animals did not show any degree of incoordination even when the liver stores of vitamin A had been nearly depleted.



Fig. 1--Cloudy condition of the cornea present in a vitamin A deficient ram.



Fig. 2--A vitamin A deficient ram showing extreme weakness.

The deficient rams that were returned to a vitamin A containing diet recovered from all lesions. However even though the vitamin A liver stores of these animals had been drastically reduced no severe symptoms were evident in most of the rams in this group at the time that these began receiving vitamin A. One of the rams receiving PMS treatment exhibited a protruding eye with evident infection. At autopsy the veterinarian diagnosed purulent pleuritis as the immediate cause of death. Another ram on the same treatment developed an abscess in the sheath area. This same ram showed bloody urine before dying.

In general the rams on thyroprotein treatment showed less severe symptoms than did other deficient animals. The appetite and growth of these rams was higher than in other deficient rams. Rams in the A⁻ + Testosterone group exhibited symptoms similar in nature to those of other rams on the vitamin A deficient diet.

At autopsy no striking gross pathological lesions were evident in rams subjected to the vitamin A deprivation.

This author believes that some of the deficiency symptoms observed were in part caused by multiple nutritional deficiencies resulting from the decrease in feed consumption observed in the vitamin A deficient rams. The A⁻ + Thyroprotein group had more appetite as well as less severity in their symptoms. It should be stated however that the deficient ration would have supplied the required amounts of nutrients had they been consumed at the same level as the control ration. The basic cause of this multiple deficiency is therefore an absence of vitamin A.

General Effects of Gamma Irradiation

Rams exposed to irradiation suffered a gradual loss in appetite until death. Those rams surviving the irradiation treatment eventually recovered from the loss of appetite.

No signs of external hemorrhaging were evident in any of the rams exposed to irradiation. Several rams exhibited limited anal bleeding prior to death. Most of the rams would lie down, shiver and show signs of pain prior to death. At autopsy multiple hemorrhages were present in the lungs and chest cavity areas. Limited hemorrhagic spots were observed in the cortical region of the kidneys and adrenals and in the urinary bladder of some rams. No characteristic histological lesion was observed in the testes of animals exposed to irradiation.

The multiple external and internal hemorrhaging described by Meacham et al. (1962) in pigs exposed to 350 roentgens of whole body gamma irradiation from a Co^{60} source were not observed in these rams suggesting different mode of action of irradiation in these two species.

Plasma and Liver Vitamin A Content

Table 4 shows plasma vitamin A values for rams in the various allotment groups (Group I, II and III) and treatments, a summary of all treatment groups is presented in Table 5. It should be noted that the various treatments appearing in Table 4 were not started until after the biopsy. However the rams appear grouped into the various treatments so that comparisons can be made on the vitamin A reserves of rams assigned to a given treatment. Liver storage of vitamin A determined on biopsy samples is also shown in Table 5.

TABLE 4

VITAMIN A PLASMA AND LIVER VALUES FOR THE VARIOUS GROUPS DURING PHASE I^a

	Ram No.	Blood, mcg./100 ml. of plasma						Liver, mcg./g. fresh liver					
		1/23	2/27	4/3	4/24	4/30	5/23	5/28	6/26	7/1	4/24	5/28	7/1
Group I													
Control	2	22	52	52	33 ^b	-	-	-	-	-	146.0	-	-
Deficient	1	16	15	12	0	-	-	-	-	-	0.6	-	-
Returned	2	20	21	10	2	-	-	-	-	-	0.7	-	-
A ⁻ + PMS	2	32	14	4	0	-	-	-	-	-	2.6	-	-
A ⁻ + Thy.	2	22	21	10	4	-	-	-	-	-	1.1	-	-
A ⁻ + Test.	2	24	16	6	3	-	-	-	-	-	.9	-	-
A ⁻ Irrad.	2	18	15	6	5	-	-	-	-	-	3.3	-	-
Control Irrad.	2	27	50	68	16	-	-	-	-	-	197.0	-	-
Group II													
Control	2	36	40	46	-	44	58	44 ^b	-	-	-	191.0	-
Deficient	2	18	16	20	-	8	3	5	-	-	-	.6	-
Returned	2	28	16	26	-	5	2	4	-	-	-	.7	-
A ⁻ + PMS*	2	24	26	38	-	15	10	14	-	-	-	2.2	-
A ⁻ + Thy.	2	36	21	15	-	12	2	2	-	-	-	.6	-
A ⁻ + Test.	2	21	24	12	-	7	3	3	-	-	-	1.7	-

TABLE 4 (Continued)

Ram No.	1/23	2/27	Blood, mcg./100 ml. of plasma					Liver, mcg./g. fresh liver		
			4/3	4/24	4/30	5/23	5/28	6/26	7/1	7/1
A ⁻ Irrad.	2	20	24	27	-	18	17	8	-	9.1
Control Irrad.	2	22	38	44	-	54	63	51	-	118.0
Group III										
Control	2	32	48	60	-	56	65	-	90	42 ^b - 125.0
Deficient	2	24	38	14	-	20	40	-	13	4 - 2.9
Returned	2	34	30	36	-	36	28	-	32	20 - 52.1
A ⁻ + PMS	2	23	28	38	-	36	33	-	16	1 - 2.2
A ⁻ + Thy.	2	21	32	30	-	27	30	-	24	4 - 4.1
A ⁻ + Test.	2	30	27	26	-	26	26	-	10	2 - 4.6
A ⁻ Irrad.	2	44	45	41	-	38	13	-	20	11 - 10.8
Control Irrad.	2	39	44	65	-	66	44	-	65	48 - 158.5

^aPhase I extends from start of experiment to reallocation to the various treatments. Each value represents the average of two rams, excepting the values expressed for the deficient in Group I where only one ram was used.

^bValues in this column represent blood levels of vitamin A at time of reallocation. Liver values for the same dates heading these columns represent liver stores at time of reallocation measured on tissue obtained by biopsy.

TABLE 5
AVERAGE VITAMIN A PLASMA AND LIVER VALUES FOR THE
VARIOUS TREATMENTS AT TIME OF REALLOTMENT

Treatment	Number of Rams	Plasma mcg./100 ml. of plasma	Liver mcg./g. of fresh liver
Control ^a	6	36	154.0
Deficient ^a	5	3	1.4
Return ^a	6	9	17.8
A ⁻ + PMS ^a	6	5	2.3
A ⁻ + Thy. ^a	6	3	1.9
A ⁻ + Test. ^a	6	3	2.4
A ⁻ + Irrad. ^a	6	8	7.7
Control Irrad. ^a	6	57	157.8
All vitamin A normal ^b	12	39	156.0
All vitamin A deficient ^c	35	5	5.6

^aValues represent an average of all three groups for a given treatment.

^bValues represent an average of all three groups for all animals receiving vitamin A in their diets.

^cValues represent an average of all three groups for all animals deprived of vitamin A in their diets.

The vitamin A reserves in all rams on the diet containing vitamin A were as expected much higher than in rams on the vitamin A deficient diet. All rams on this latter diet had received similar diets since June 1962; however the drop in plasma vitamin A took place at differing times. The different initial vitamin A reserves in these rams no doubt accounts for most of this variability.

Table 4 shows that the variability in plasma vitamin A occurring from month to month in a given pair of rams was considerably reduced when the plasma vitamin A levels had been drastically diminished, at this latter stage a plateau is reached. It was at this time that the biopsy was performed to ascertain the deficiency condition. In general vitamin A liver reserves were at abnormally low levels at time of assignment of the rams on the deficient diet to the various treatments in phase II. The rams assigned to the returned treatment in group III showed higher vitamin A reserves than other deficient rams but considerably lower than those present in the controls or normal irradiated rams. In general one can state that a vitamin A deficiency condition was present at the time of assignment of the rams on the deficient diet to the various treatments in phase II. It should be kept in mind however that the point at which the liver vitamin A values shown in Table 5 were reached in these rams is unknown, since it is generally agreed that a poor correlation exists between plasma and liver vitamin A values. The only possible way to ascertain when the low liver vitamin A storages found at time of reallocation were reached, would have been to perform a series of biopsies on the same individual rams throughout the experiment. This is not feasible since the stress would

most likely have resulted in heavy death losses. It is also possible to slaughter random samples at different periods and based on these samples infer vitamin A status of the population in question. However, in this experiment the latter method could not be followed since only a limited number of rams were available.

Table 18 of the Appendix shows individual ram plasma and liver vitamin A reserves at time of reallocation. Also in Table 18 are individual final plasma vitamin A values determined on rams surviving to termination of the experiment. The plasma vitamin A values found present in rams on the vitamin A deficient diet were very low and much lower than those in the control group. Rams in the returned group had final plasma vitamin A values comparable to the controls.

Feed Intake

Feed intake data for individual rams were not possible to record because facilities used in this experiment did not provide for individual allotment. During phase II feed intake data were further confounded because the assignment of rams to one of the 8 treatments was done in three stages so that each pen held increasingly larger numbers of rams until the 47 were assigned a treatment. Thus the same number of rams was not present at all times in a pen chosen for one of the 8 treatments. For these reasons statistical analysis of feed intake data were not possible. However some trends were in evidence so that some conclusions can be drawn from the available data.

Table 6 shows feed intake data for the various treatment groups during phase I and II. In phase I rams on the control and vitamin A

TABLE 6

DAILY GAIN AND FEED INTAKE FOR RAMS
DURING EXPERIMENTAL PERIOD

Treatment	No. of rams	Average Daily Gain, lb.		Average Feed Intake, lb.			
		1st phase	2nd phase combined	1st phase	2nd phase combined		
Control	6	.24	0	.11	3.0	2.1	2.6
Deficient	5	.06	-.10	-.04	1.8	.85	1.33
Returned	6	.10	.11	.11	1.8	1.90	1.85
A ⁻ + PMS	6	.05	-.04	-.03	1.8	.92	1.36
A ⁻ + Testosterone	6	.04	-.27	-.07	1.8	.71	1.26
A ⁻ + Thyroprotein	6	.06	-.04	.01	1.8	1.59	1.69
A ⁻ + Irradiated	6	.06	-.23	-.01	1.8	.67	1.24
Control Irradiated	6	.23	-.23	.16	3.0	1.54	2.27

^aFirst phase extended from start of experiment to assignment to the various treatments.^bSecond phase extended from reallocation to termination or death.^cCombined represents an average figure from start of experiment to termination or death.

deficient diet consumed an average of 3.0 and 1.8 lbs. per head daily, respectively. During phase I no widespread occurrence of vitamin A deficiency symptoms was observed in the rams on the vitamin A deficient diet indicating that the detrimental effect of vitamin A deprivation on feed intake was manifested prior to other external signs of a vitamin A deficient condition.

During phase II average daily feed intake per head was 2.1, .85, 1.90, .92, .71, 1.59, .67 and 1.54 lbs. for the control, deficient, returned, A⁻ + PMS, A⁻ + Testosterone, A⁻ + Thyroprotein, A⁻ + Irradiated and Control Irradiated groups respectively. Rams receiving the vitamin A containing diet during phase II (control, returned and control irradiated groups) consumed more feed than rams on the other treatments, except for the A⁻ + Thyroprotein group. It is interesting to note that the control group consumed less feed during phase II. This resulted from the increased stress to which all rams were subjected during this phase. Thus the decrease in feed consumption evident in rams on the vitamin A deficient diet can be attributed partially to the increased stress. The A⁻ + Thyroprotein group consumed more feed than other rams on the vitamin A deficient diet. PMS or testosterone treatment did not have any beneficial effect on the appetite of vitamin A deficient rams. Irradiation appeared to have had a depressing effect on feed intake on all rams irradiated.

Feed consumption in the control group was comparable to that reported by Meacham (1962) but somewhat less than that of Tilton (1962). The latter used corn cobs as a source of fiber in a diet very similar to that used in this experiment. Meacham (1962) used Solka-Floc as the source of fiber which is similar to that used in this experiment. At slaughter,

extensive amount of wool was found in the rumen of rams in all treatments. This was to be expected from the frequent "wool eating" observed in the experimental rams. This suggests that the amount or the source of fiber provided or both were not adequate. The possibility also exists that some other nutritional factor was not adequately provided.

Body Weight Changes

Table 6 shows average daily gains during phases I, II and I and II combined. In phase I rams had not been assigned to the various treatments; however for the purposes of this discussion rams that were assigned to a treatment during phase II appear in this treatment in phase I, so that it is possible to follow growth rate for rams in a group prior to and during treatment.

During phase I average daily gains were .24, .06, .10, .05, .04, .06 and .23 lbs. for the control, deficient, returned, A⁻ + PMS, A⁻ + Testosterone, A⁻ + Thyroprotein, A⁻ + Irradiated and Control Irradiated groups respectively. Table 7 shows the ranked average daily gains for the various treatment groups. Rams on the vitamin A supplemented diet gained significantly more than those on the vitamin A deficient diet. As was to be expected the various groups on the vitamin A deficient diet did not differ significantly between them. Individual daily gains and weights during phase I are shown in Tables 19 and 20.

Average daily gains for the various treatment groups during phase II as shown in Table 6 were 0, -.10, .11, -.04, -.27, -.04, -.23, -.23 lbs. for the control, deficient, returned, A⁻ + PMS, A⁻ + Testosterone, A⁻ + Thyroprotein, A⁻ Irradiated and Control Irradiated groups, respectively.

TABLE 7

DIFFERENCES IN DAILY GAIN FOR THE VARIOUS TREATMENTS

Phase	Ranked Means for the Various Treatments ^a							
	Control	Control Irradiated	Returned	Deficient	A ⁻ + Irradiated	A ⁻ + Thyroprotein	A ⁻ + PMS	A ⁻ + Testosterone
Phase I	.24	.23	.10	.06	.06	.06	.05	.04
Phase II	Returned	Control	A ⁻ + PMS	A ⁻ + Thyroprotein	Deficient	A ⁻ + Irradiated	Control Irradiated	A ⁻ + Testosterone
	.11	0	-.04	-.04	-.10	-.23	-.23	-.27
Combined	Control Irradiated	Control	Returned	A ⁻ + Thyroprotein	A ⁻ + Irradiated	A ⁻ + PMS	Deficient	A ⁻ + Testosterone
	.16	.11	.11	.01	-.01	-.03	-.04	-.07

^aMeans underscored by the same line are not significantly ($P < .05$) different.

Table 7 shows the ranked average daily gains for the same groups. The returned group gained significantly more than all other treatment groups except for the controls. The controls did not gain significantly more than the A⁻ + PMS, A⁻ + Thyroprotein and deficient groups. The low average daily gains in the control rams is largely due to the drastic weight reduction of ram No. Y43 who lost 45 lbs. during phase II. Rams exposed to irradiation had very low average daily gains. The depressing effect of irradiation on average daily gains was equivalent on both control and A⁻ irradiated groups. Individual weights and average daily gains are shown in Tables 19 and 20 of the Appendix. The combined phase I and II average daily gains are shown in Table 6. Table 7 shows the ranked average daily gain for the various treatment groups during phase I and II combined. All rams on the vitamin A supplemented diet gained significantly more than rams on the deficient diet. Table 19 in the Appendix shows individual average daily gains during phases I and II combined.

Figures 3, 4 and 5 illustrate the body weight change in rams on the various treatments. Before discussing these figures it should be noted that the number of rams per treatment was small from the start (6 per group) and the heavy death loss inflicted by the vitamin A deficiency drastically reduced the rams per group so that in many instances the values plotted for a given treatment represent the body weight change of a very limited number of rams. It is true however, that rams that died before termination of the experiment were those who had undergone the most drastic weight loss so that the slope of the plotted line would have been steeper had they survived.

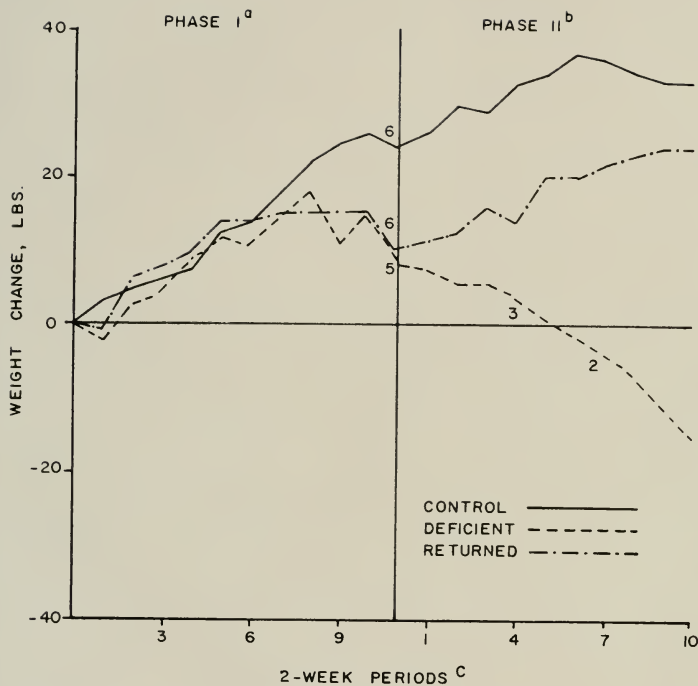


Fig. 3--Effect of vitamin A supplementation on body weight changes of rams.

^aPhase I extended from the start of experiment to assignment to the various treatments. From periods 1 to 6 there were 6 rams per group. From periods 7 to 8 there were 4 and from periods 9 to 11, 2 rams were present per group.

^bPhase II extended from assignment to the various treatments to termination of the experiment.

^cNumbers adjacent to plotted values represent animals remaining in each group for a given two week period. Differences in numbers between periods in a given group resulted from death loss.

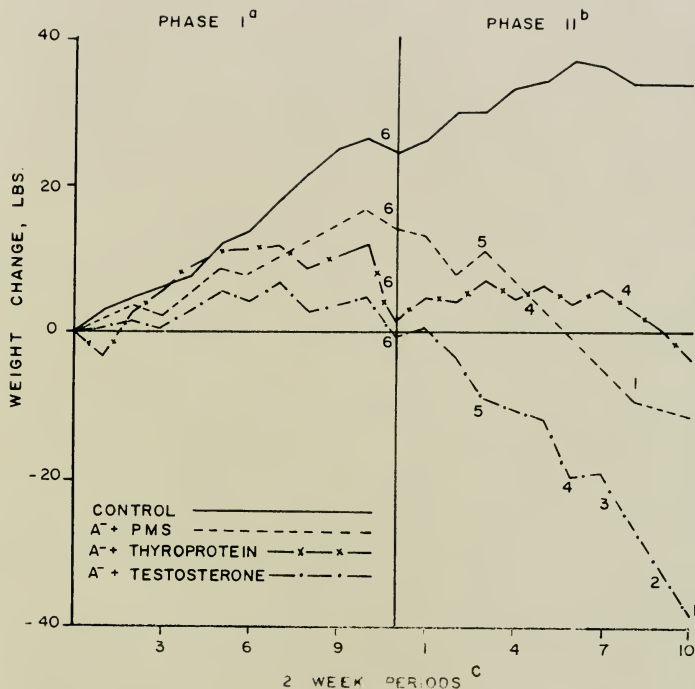


Fig. 4.--Effect of various hormone treatments on body weight changes of vitamin A deficient rams.

^aPhase I extended from the start of experiment to assignment to the various treatments. From periods 1 to 6 there were 6 rams per group. From periods 7 to 8 there were 4 and from periods 9 to 11, 2 rams were present per group.

^bPhase II extended from assignment to the various treatments to termination of the experiment.

^cNumbers adjacent to plotted values represent animals remaining in each group for a given two-week period. Differences in numbers between periods in a given group resulted from death loss.

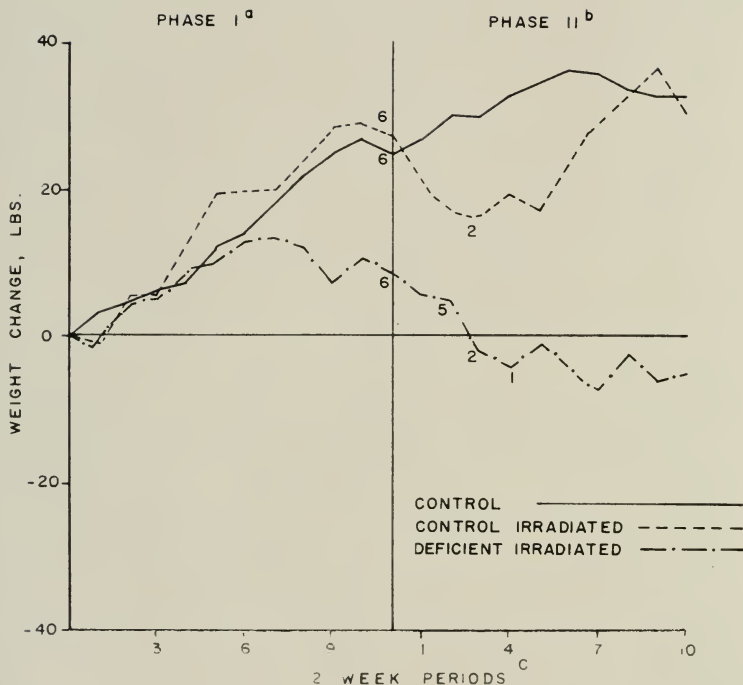


Fig. 5.--Effect of irradiation on body weight changes of vitamin A deficient and normal rams.

^aPhase I extended from the start of experiment to assignment to the various treatments. From periods 1 to 6 there were 6 rams per group. From periods 7 to 8 there were 4 and from periods 9 to 11, 2 rams were present per group.

^bPhase II extended from assignment to the various treatments to termination of the experiment.

^cNumbers adjacent to plotted values represent animals remaining in each group for a given two week period. Differences in numbers between periods in a given group resulted from death loss.

Figure 3 illustrates the body weight changes for the control, deficient, and returned groups. During phase I the body weight change experienced by all rams was similar up to the 14th week (period 8). At this point the control rams continued to gain while rams on the deficient diet began to loose weight. During phase II the rapid recovery experienced by the returned rams is clearly shown. The deficient rams continued to loose weight while the controls increased in weight up to period 6 and then reached a plateau. Figure 4 shows the body weight change for the control and hormone treated groups. During phase I the significant increase in body weight in the control rams compared to rams on the deficient diet is clearly shown. During phase II rams in the A⁻ + PMS and A⁻ + Testosterone groups suffered a drastic weight reduction. It is interesting to note the beneficial effect thyroprotein had on preventing body weight loss up to period 7. This was partially due to a stimulation in appetite produced by the thyroprotein.

Figure 5 illustrates the effects of irradiation on⁷ body weight changes. It is clear that all rams exposed to irradiation suffered severe weight losses. This correlates well with the decrease in feed consumption observed in the same rams. Those rams in the control irradiated group who survived irradiation recovered.

The depressing effect on growth normally associated with vitamin A deficiency was observed in this experiment. Rams whose vitamin A reserves had been drastically reduced and were experiencing severe weight losses were able to recover when 3,000 I.U. of vitamin A per lb. of feed were provided. PMS and testosterone treatment did not prevent further weight losses in vitamin A deficient rams. Thyroprotein appeared to have a stimulating effect on appetite resulting partially in less severe weight

losses, as compared to other rams on the same vitamin A deficient diet without thyroprotein. This finding would appear to contradict the widely accepted theory that an increase in appetite which would normally stimulate growth, if the diet in question is deficient in a nutrient would accelerate needs for the nutrient in question. This would in turn increase severity of deficiency symptoms. Irradiation affected feed intake almost immediately. This was accompanied by a severe weight loss until death. The appetite of rams surviving irradiation returned to normal and did not undergo further weight reduction.

Analysis of variance for differences in daily gains between the various treatments in phases I, II and I and II combined are shown in the Appendix Tables 26, 27, and 28 respectively.

Survival

Average survival time expressed in days as shown in Table 8 was. 141, 82, 141, 85, 97, 125, 46 and 63 for rams in the control, deficient, returned, A⁻ + PMS, A⁻ + Testosterone, A⁻ + Thyroprotein, A⁻ irradiated and control irradiated groups respectively. Survival time for rams in the various allotment groups (I, II and III) was also shown in Table 8. Per cent death loss for the same groups as shown in Table 9 was 0, 80, 0, 84, 66, 50, 84, and 66. Table 10 shows the ranked average survival time in days for rams in the various treatment groups.

Rams in the control and returned groups suffered no death loss. They survived a significantly larger number of days than rams on the other treatments except for those on thyroprotein. This latter group survived significantly more days than all remaining groups except for rams in the

TABLE 8

AVERAGE SURVIVAL TIME FOR RAMS IN THE VARIOUS TREATMENTS^a

Treatment	1st Group ^b		2nd Group ^c		3rd Group ^d		Combined	
	No. of Animals	Days	No. of Animals	Days	No. of Animals	Days	No. of Animals	Days
Control	2	141	2	141	2	141	6	141
Deficient	1	141	2	90	2	45	6	82
Return	2	141	2	141	2	141	6	141
A ⁻ + PMS	2	52	2	102	2	103	6	85
A ⁻ + Testosterone	2	133	2	51	2	107	6	97
A ⁻ + Thyroprotein	2	141	2	137	2	97	6	125
A ⁻ Irradiated	2	83	2	23	2	31	6	46
Control Irradiated	2	85	2	25	2	81	6	63
Average	2	115	2	89	2	93	6	99

^aExpressed in days of survival from reallotment to slaughter or death.^bRams in this group were started on phase II on April 29, 1963 and were slaughtered September 17, 1963.^cRams in this group were started on phase II on June 10, 1963 and were slaughtered October 29, 1963.^dRams in this group were started on phase II on July 15, 1963 and were slaughtered December 3, 1963.^eThis represents the average survival time for the three groups.

TABLE 9
PER CENT DEATH LOSS FOR RAMS IN THE
VARIOUS TREATMENTS

Treatment	No. of Animals		Per cent Death Loss
	Initial	Final	
Control	6	6	0
Deficient	5	1	80
Returned	6	6	0
A ⁻ + PMS	6	1	84
A ⁻ + Testosterone	6	2	66
A ⁻ + Thyroprotein	6	3	50
A ⁻ Irradiated	6	1	84
Control Irradiated	6	2	66

TABLE 10
DIFFERENCES IN SURVIVAL TIME BETWEEN
THE VARIOUS TREATMENTS

Ranked Means for the various Treatments							
Control	Return	A ⁻ + Thyro- protein	A ⁻ + Tes- tosterone	A ⁻ + PMS	Deficient	Control Irradiated	A ⁻ Irradiated
141	141	125	97	85	82	63	46

^aMeans underscored by the same line are not significantly ($P < .05$) different.

A⁻ + Testosterone group. There was no difference in tolerance to gamma irradiation between the control and deficient groups exposed to irradiation as measured by survival time. Differences in survival time between allotment groups (I, II and III) were not significant.

Most of the rams whose vitamin A reserves had been depleted eventually died. Treatment with either testosterone or PMS was not effective in preventing death losses resulting from a vitamin A deficiency. Daily capsules of thyroprotein were effective in reducing death loss of vitamin A deficient rams. Rams whose vitamin A reserves had been drastically reduced but were replenished by feeding 3,000 I.U. of vitamin A per lb. of feed daily suffered no death loss. The vitamin A status of rams exposed to irradiation did not affect significantly the survival time of these animals.

Individual survival time for all rams is shown in Appendix Table 21. Analysis of variance for differences in survival time between the various treatment groups is shown in the Appendix Table 28.

Semen Characteristics

Semen data were statistically analyzed during the first ten weeks of phase II. Beyond this point total number of rams per group was too limited for statistical consideration of the data.

A series of figures are included which illustrate semen characteristics of rams in the various treatments during all of phase II. It should be noted that in some instances the number of rams per treatment, as indicated by the numbers in parentheses adjacent to the plotted values, is small resulting from heavy death losses in those groups. It is also

true however that death losses in treatment groups on the vitamin A deficient diet occurred when these rams were in advanced stages of avitaminosis A. At this time changes in semen characteristics affected by vitamin A deficiency had taken place. So that in many instances plotted lines, even though based on data from a reduced number of rams, do reflect the trend of the initial population. For example Figure 10 illustrates per cent sperm motility for several treatments. In looking at the A⁻ + Testosterone group one sees that from weeks 11 to 12, 2 rams died. After week 12 a drop in per cent motility was observed. Appendix Table 24 shows individual semen characteristics for all weeks. One sees that the 2 rams that died were not showing high sperm motility, so that the drop in per cent sperm motility at the twelfth week was not due to death of rams with high sperm motility values but rather to a drop in values in surviving rams. Had the rams survived the change from weeks 12 to 13 the slope of the line would have been more pronounced. This should be kept in mind in drawing conclusions from the experimental data available.

A summary of semen data collected monthly during phase I is shown in Table 11. Per cent abnormal sperm cells was not determined during phase I nor was semen data from phase I analyzed statistically.

Volume

Average semen volume for rams in all treatment groups during phases I and II as shown in Table 11 were 2.2, 1.4; .8, .9; .6, 1.1; 1.0, 1.0; .8, .5; 1.1, .7; 1.9, .9; and 1.0, .8 ml. for the control, deficient returned, A⁻ + Thyroprotein, A⁻ + PMS, A⁻ + Testosterone, control irradiated and

TABLE 11
AVERAGE SEMEN VOLUME, MOTILITY, ABNORMAL SPERM CELLS AND TOTAL SPERM CELLS
EJACULATED FOR ALL TREATMENTS DURING PHASES I AND II

Treatment Group	Semen Volume (ml.)		Per cent Motile sperm cells		Per cent Abnormal sperm cells ^a		Total sperm cells ejaculated (x10 ⁶)	
	I	II	I	II	I	II	I	II
Control	2.2	1.4	71	72	19		2.945	1723
Deficient	.8	.9	29	22	50		.637	461
Returned	.6	1.1	66	68	18		.605	1184
A ⁻ + Thyroprotein	1.0	1.0	84	48	36		1.627	1163
A ⁻ + PMS	.8	.5	57	19	37		1.025	234
A ⁻ + Testosterone	1.1	.7	88	18	33		1.365	313
Control Irradiated	1.9	.9	78	44	46		3.036	1346
A ⁻ Irradiated	1.0	.8	83	46	37		1.421	670

^aPer cent abnormal sperm cells was not determined during phase I.

A⁻ irradiated groups respectively. Rams on the vitamin A containing diet (control and control irradiated groups) showed twice⁷ as much semen volume as those on the vitamin A deficient diet during phase I. From phase I to phase II there was a drop in average semen volume in the control rams. This was probably due to the increased frequency of collections during phase II. The deficient group did not show a drop in average semen volume during phase II. The returned group increased semen volume during phase II. This change could be attributed to commencement of vitamin A supplementation. Figure 6 illustrates semen volume changes for these 3 groups during phase II. Notice how the control rams always maintained semen volume values above the other 2 groups. The gradual recovery of the returned group is clearly shown. Of the hormone supplemented groups the PMS and testosterone treated rams suffered a further reduction in average semen volume during phase II. Thyroprotein treatment did not have this harmful effect on semen volume. Figure 7 illustrates semen volume changes for the hormone treated groups compared to the controls. Semen volume in the testosterone group was quite variable from weeks 12 to 19 but it remained lower than in the controls. The PMS treated group had low semen volume throughout phase II. The semen volume values for the thyroprotein groups did not drop below their initial value in any of the 20 weeks. Both groups of irradiated rams showed reduced average semen volume during phase II; this change was more drastic in the control irradiated group. Figure 8 shows this change during phase II.

It should be kept in mind that semen volume differences between all treatments were not of statistical significance when analyzed. Tables 30 through 39 show analysis of variance for differences in semen volume

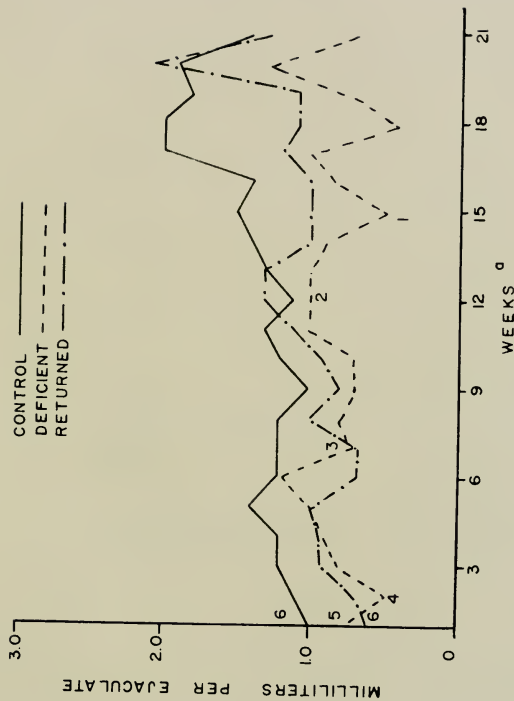


Figure 6.--Effect of vitamin A supplementation on mean semen volume of rams.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. Differences in numbers between weeks in a given group resulted from death loss.

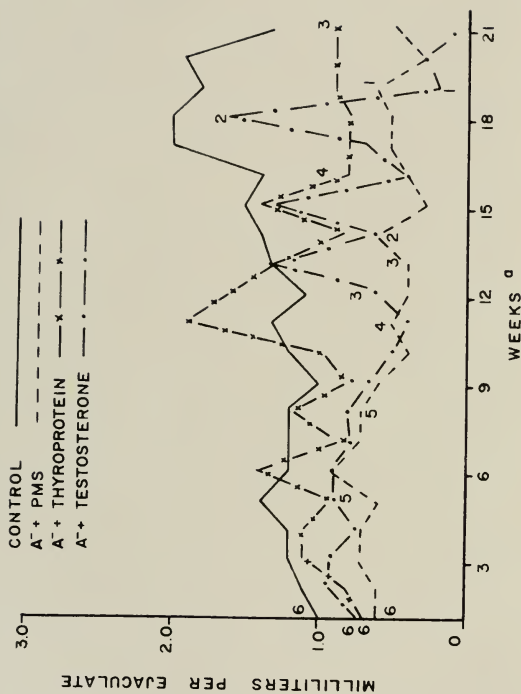


Figure 7.--Effect of various hormone treatments on mean semen volume of vitamin A deficient rams.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. Differences in numbers between weeks in a given group resulted from death loss.

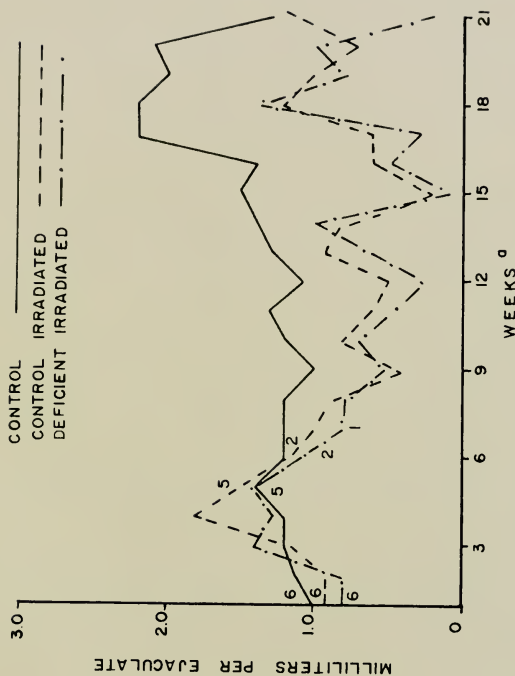


Figure 8.--Effect of irradiation on mean semen volume of vitamin A deficient and normal rams.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. Differences in numbers between weeks in a given group resulted from death loss.

during the first 10 weeks of phase II. However some trends were evident enough to permit certain conclusions. Vitamin A deficiency appeared to have had a depressing effect on average semen volume. This effect could not be overcome with thyroprotein treatment. Treatment with PMS or testosterone resulted in a more pronounced drop in semen volume of the vitamin A deficient rams. Irradiation appeared to have had a harmful effect on average semen volume from normal and vitamin A deficient rams. The reduction in semen volume resulting from a vitamin A deficiency found in this experiment is in agreement with the findings of Lindley et al. (1949). They also found no beneficial or harmful influence on semen volume of vitamin A deficient rams from treatment with PMS or testosterone. Goswami (1962) reported a reduction in semen volume of bulls treated with thyroprotein during the summer months in India. This effect on semen volume was not evident in thyroprotein treated vitamin A deficient rams in the study reported. Black et al. (1950) reported that thyroprotein had no effect on semen volume in rams which is in agreement with our findings. However it is very difficult to compare our data with those reported elsewhere because of dissimilarities in climate, diets, hormones dosage and age of rams present.

The harmful effects of irradiation on semen volume apparent in this study have not been reported elsewhere.

Individual semen volume values for all rams during phase II are shown in the Appendix Table 24.

Motility

Average per cent motile sperm cells during phases I and II as shown in Table II were 71, 72; 29, 22; 66, 68; 84, 48; 57, 19; 88, 18;

78, 44; and 83, 46 for the control, deficient, returned, A⁻ + Thyroprotein, A⁻ + PMS, A⁻ + Testosterone, control irradiated and A⁻ irradiated groups respectively. During phase I the control group showed motility values higher than rams in the deficient group. Motility values in other groups on the vitamin A deficient diet were comparable to the controls. Average motility values for the control rams were similar in phases I and II. The deficient group suffered further loss in motility values during phase II. Average motility for the returned group had not been affected during phase I so it was not possible to show whether a drop in motility from a vitamin A deficiency can be overcome by vitamin A supplementation.

Figure 9 illustrates changes in per cent motile sperm during phase II, in the control, deficient and returned groups. Except for week 19 motility values for the deficient group were lower than those of the control and returned groups. Differences in per cent motile sperm between the control and deficient group as shown in Table 12 were significant in weeks 8, 9 and 10 only. Analysis of variance for differences in per cent motile sperm during all of the first 10 weeks of phase II are shown in the Appendix Tables 30 through 39. Average per cent motile sperm values as shown in Table 11 were lowered during phase II in the hormone treated rams. Thyroprotein treatment overcame somewhat the depressing effects on motility from the vitamin A deficiency. Figure 10 illustrates average per cent motile sperm for the hormone treated and control groups during phase II. From weeks 1 to 4 a drop in average per cent motile sperm cells was observed in all hormone treated groups. From weeks 4 to 11 average motility was variable in the A⁻ + Testosterone group but eventually dropped to 0 from weeks 12 to 21. From weeks 4 to 16 average per cent motile sperms remained

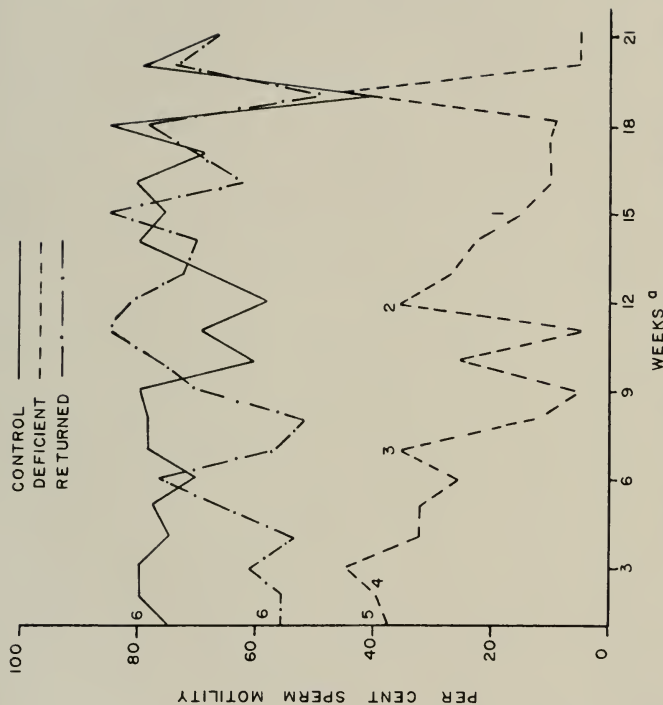


Figure 9.--Effect of vitamin A supplementation on mean sperm motility of rams.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. Differences in numbers between weeks in a given group resulted from death loss.

TABLE 12

COMPARISONS BETWEEN THE VARIOUS TREATMENT GROUPS FOR DIFFERENCES
IN PER CENT MOTILE SPERM CELLS--WEEKS 8, 9 AND 10

Treatment Groups	Week		
	8	9	10*
Control versus Deficient	.01	.05	.05
Control Irradiated versus Deficient Irradiated	-	-	-
Control and Deficient versus Control Irradiated and Deficient Irradiated.	-	-	-
A ⁻ + Thyroproteine versus A ⁻ + PMS	-	-	-
A ⁻ + Thyroproteine and A ⁻ + PMS versus A ⁻ + Testosterone	-	-	-
Returned versus A ⁻ + Thyroproteine and A ⁻ & PMS and A ⁻ + Testosterone	-	.05	.05
Control and Deficient and Control Irradiated and Deficient Irradiated versus All Others	-	-	-

*P < .05

P < .01

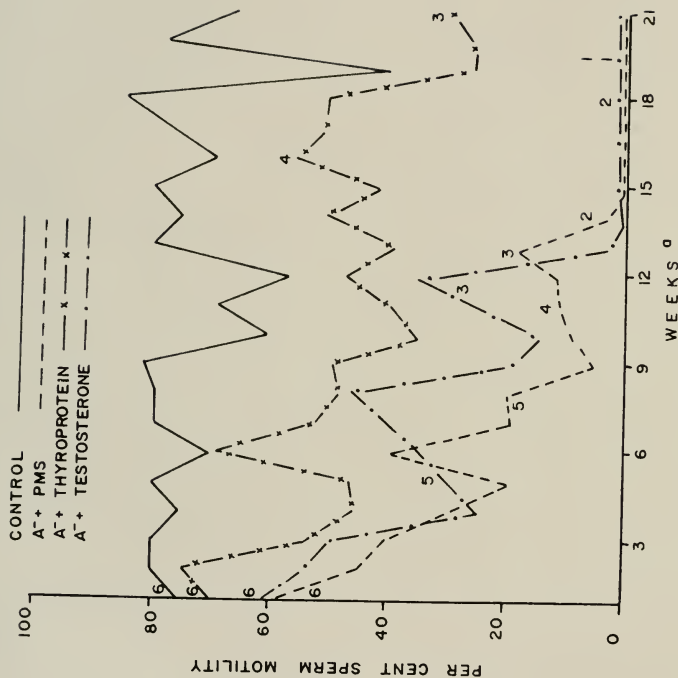


Figure 10.--Effect of various hormone treatments on mean sperm motility of vitamin A deficient rams.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. Differences in numbers between weeks in a given group resulted from death loss.

more or less at the same level in the A⁻+thyroprotein group. The A⁻ + PMS group showed a gradual loss of sperm motility from weeks 1 to 14, at this point a 0 value was reached which remained to termination of the experiment. As shown in Table 12 differences in per cent motile sperm between the returned (which had motility values comparable to the control group) and the hormone treated groups were statistically significant at weeks 9 and 10. As shown in Table 11 average per cent motile sperm was lower in phase II for both groups irradiated. In the A⁻ irradiated group sperm motility values were not as low as in other groups on the vitamin A deficient diet. Figure 11 illustrates changes in motility values in the control and irradiated groups. The tremendous variability in per cent sperm motility in the A⁻ irradiated group can be attributed mostly to the fact that only one ram survived from weeks 7 to 21. In general per cent sperm motility was lower in the irradiated groups than in the control throughout most of phase II. As shown in Table 12 differences in per cent sperm motility between the irradiated groups and their counterparts (control and deficient groups) were not statistically significant in any of the first 10 weeks of phase II. Analysis of variance for differences in per cent sperm motility during phase II are shown in the Appendix, Tables 30 through 39.

Results obtained in this experiment indicate that sperm motility is severely affected by a vitamin A deficiency. This lowered sperm motility could not be improved by treatment with PMS or testosterone. Thyroprotein alleviated the depressing effect of vitamin A deficiency on sperm motility. This supports the findings of Lindley et al. (1949). They were unsuccessful in preventing or improving low sperm motility in vitamin A deficient rams by treatment with testosterone or PMS. Gunn et al. (1942)

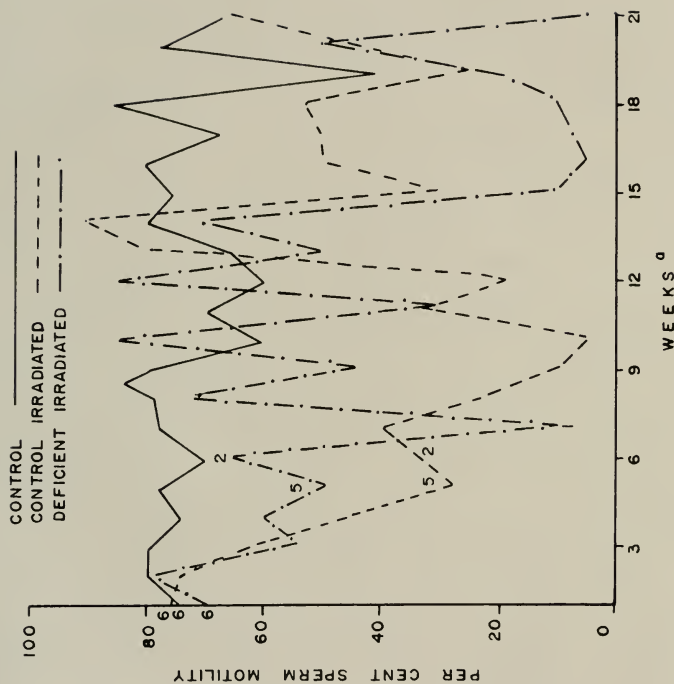


Figure 11--Effect of irradiation on mean sperm motility of vitamin A deficient and normal rams.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. Differences in numbers between weeks in a given group resulted from death loss.

improved sperm motility of a vitamin A deficient ram by injecting PMS. Hogdson et al. (1946) obtained similar results in a vitamin A deficient bull. The discrepancy in results between the results of the latter two authors and those of Lindley et al. (1949) and ours can not be emphasized since only a very limited number of experimental animals were used by Gunn et al. (1942) and Hogdson et al. (1946). The detrimental effects of irradiation on sperm motility was equivalent on both normal and vitamin A deficient rams. However sperm motility was not sufficiently reduced to suppose a serious impairment to the reproductive capacity of these rams.

Individual per cent motility for all rams throughout phase II of the experiment is shown in the Appendix, Table 23.

Per Cent Abnormal Sperm

As shown in Table 11 per cent abnormal sperm values were 19, 50, 18, 36, 37, 33, 46, and 37 for the control, deficient, returned, A⁺ + Thyroprotein, A⁺ + PMS, A⁺ + Testosterone, control irradiated and A⁺ irradiated groups respectively. It is apparent from these data that groups on the vitamin A deficient diet showed a higher percentage of abnormal sperms in their semen. Figure 12 illustrates changes in per cent abnormal sperm values for the control, deficient and returned groups during phase II. The control group maintained per cent abnormal sperm at a low level throughout the 21 weeks of phase II. In the fourth week rams in the deficient group began to show an increasingly larger percentage of abnormal sperm while the opposite occurred in the returned group. Differences in percentage of abnormal sperm between these 3 groups were not significant statistically but they appeared to be of biological importance.

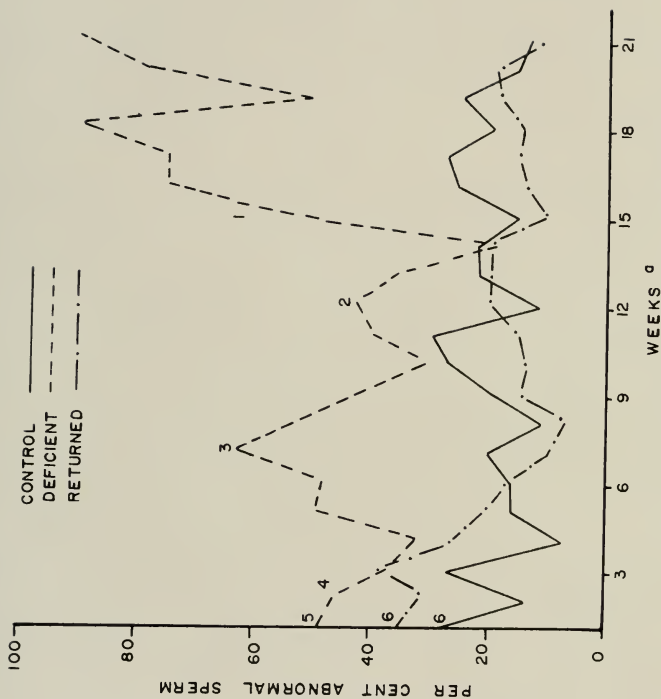


Figure 12.--Effect of vitamin A supplementation on per cent abnormal sperm of rams.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. Differences in numbers between weeks in a given group resulted from death loss.

As shown in Table 11 it appeared that treatment with testosterone, PMS or thyroprotein was not effective in overcoming the harmful effect of vitamin A deficiency on sperm abnormality in rams, rather it appeared that hormone treatment had a detrimental effect. Figure 13 illustrates changes in percentage of abnormal sperm during phase II in the hormone treated and control groups. The A^- + PMS group had a higher percentage of abnormal sperm than the control throughout phase II. The thyroprotein treated group showed a temporary improvement in sperm abnormality percentages from weeks 3 to 10, from then on an increase in sperm abnormality was evident. Testosterone treatment produced a gradual decline in sperm abnormality from weeks 4 to 7. From weeks 7 to 12 values were variable. After week 12 no further determination of per cent abnormal sperm was possible because of the reduced sperm concentration present in the semen of these rams. These phenomena occurred in the A^- + PMS group from weeks 18 to 21. Differences in per cent abnormal sperm between the returned group (with comparable values to the control group) and the hormone treated groups were not of statistical significance. Irradiation appeared to have increased sperm abnormality. As shown in Table 13 this effect was of statistical significance during weeks 6, 7 and 8 of phase II. However only 2 rams were present in each of the irradiated groups at this time. Table 13 also shows that the effects of irradiation on sperm abnormality were more drastic during weeks 6 and 8 on the control irradiated rams. Figure 14 illustrates changes in per cent abnormal sperm values for the control and both irradiated groups throughout phase II.

Analyses of variance for differences between treatments in per cent abnormal sperm during the first 10 weeks of phase II are shown in the Appendix Tables 30 through 37.

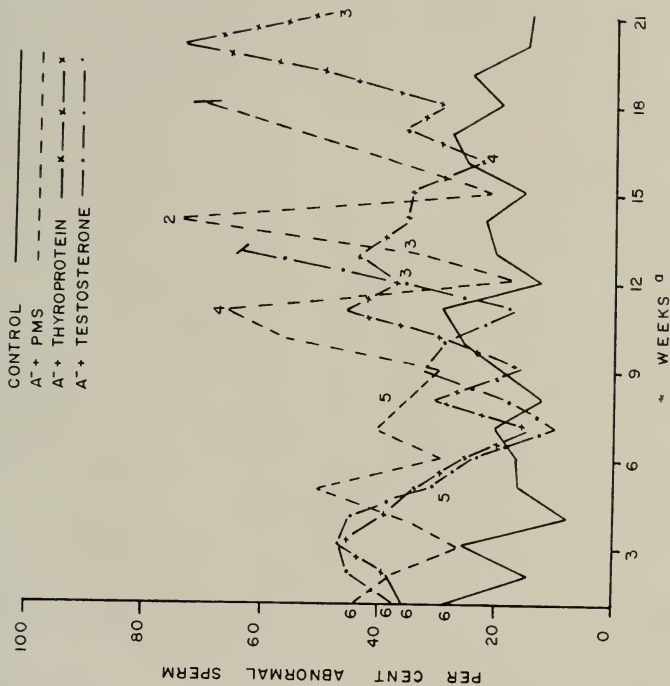


Figure 13.--Effect of various hormone treatments on per cent abnormal sperm of vitamin A deficient rams.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. Differences in numbers between weeks in a given group resulted from death loss.

TABLE 13

COMPARISONS BETWEEN THE VARIOUS TREATMENT GROUPS FOR DIFFERENCES
IN TOTAL ABNORMAL SPERM CELLS--WEEKS 6, 7 AND 8

Treatment Groups	Week		
	6	7	8
Control versus Deficient	-	-	-
Control Irradiated versus Deficient Irradiated	.01**	-	.05*
Control and Deficient versus Control Irradiated and Deficient Irradiated	.05*	.01**	.01**
A ⁻ + Thyroprotein versus A ⁻ + PMS	-	-	-
A ⁻ + Thyroprotein and A ⁻ + PMS versus A ⁻ + Testosterone	-	-	-
Returned versus A ⁻ + Thyroprotein and A ⁻ + PMS and A ⁻ + Testosterone	-	-	-
Control and Deficient and Control Irradiated and Deficient Irradiated versus all others	.05*	.01**	.05*

*p < .05

**p < .01

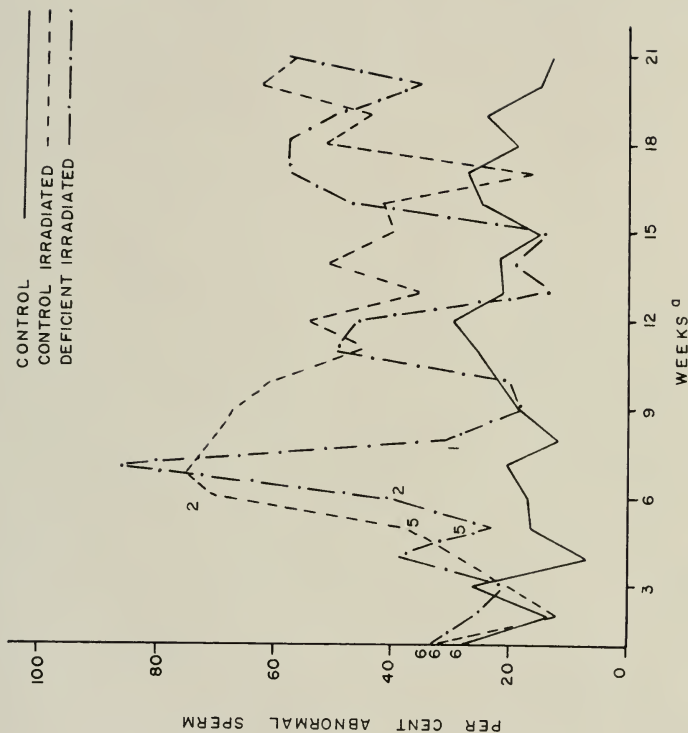


Figure 14.--Effect of irradiation on per cent abnormal sperm of vitamin A deficient and normal rams.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. Differences in numbers between weeks in a given group resulted from death loss.

Results obtained in this experiment indicate that vitamin A deficiency increased the presence of abnormal sperm in the semen of rams on a vitamin A deficient diet. This effect could not be overcome with treatment of testosterone, PMS or thyroprotein. Figure 15 shows a broken tail condition characteristic of sperm from vitamin A deficient rams. Results obtained in this study are in agreement with those reported by Lindley et al. (1949) in rams, and Bratton et al. (1948), Erb et al. (1947) and Guilbert and Hart (1935) in bulls.

Total Sperm per Ejaculate

As shown in Table 11 total sperm per ejaculate ($\times 10^6$) during phases I and II were 2945, 1723; 637, 461; 605, 1184; 1627, 1163; 1025, 234; 1365, 313; 3036, 1346; and 1421, 670 for the control, deficient, returned, A⁻ + Thyroprotein, A⁻ + PMS, A⁻ + Testosterone, control irradiated and A⁻ irradiated groups respectively. During phase I total sperm production was somewhat less in rams on the vitamin A deficient diet. The deficient group had a further drop in sperm production during phase II. A similar trend was observed in the controls. However the returned group experienced a recovery during phase II. Figure 16 illustrates changes in total sperm production for the control, deficient and returned groups. The almost immediate increase in total sperm per ejaculate in the returned group occurred from the third week on. The deficient group maintained a low sperm production throughout the 21 weeks of phase II.

As shown in Table 11 the PMS and Testosterone treated rams underwent a further drop in sperm production during phase II. The total sperm per ejaculate of rams in these two groups was slightly lower than in the



Figure 15.--Semen from vitamin A deficient rams.

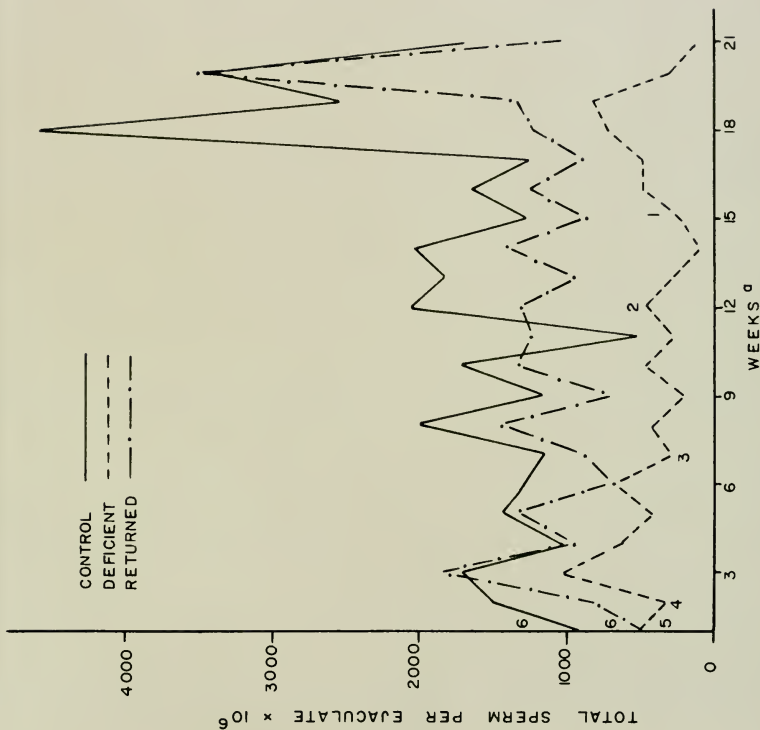


Figure 16.--Effect of vitamin A supplementation on total sperm production of rams.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. [Differences in numbers between weeks in a given group resulted from death loss.

deficient group, suggesting the possibility of a detrimental effect from treatment with these two hormones. The thyroprotein group maintained an average sperm production that was above averages found in any of the groups on the vitamin A deficient diet. Figure 17 illustrates total sperms per ejaculate from rams on the hormone treated groups throughout phase II. The rapid decrease in sperm production in testosterone and PMS treated rams is clearly shown. The beneficial effect from thyroprotein treatment on total sperm production was apparent until week 20. Irradiation caused a drop in total sperms per ejaculate of rams in both groups irradiated. This effect was more severe in the A⁻ irradiated group. Figure 18 illustrates total sperms per ejaculate from rams in the control and irradiated groups. There was a decrease in sperm production in both irradiated groups up to week 4. Froms weeks 4 to 7 sperm production in both irradiated groups was comparable to the control, at this time a decreasing trend began which remained with the A⁻ irradiated group until termination of the experiment, while the normal irradiated group appeared to have recovered in week 17.

Differences in total sperms per ejaculate between the various treatment groups were not statistically significant. However some biological differences were evident. Vitamin A deficiency had a severe effect on total sperms per ejaculate. This detrimental effect could not be overcome by treatment with testosterone or PMS. This supports the findings of Lindley et al. (1949) and is in disagreement with the results of Gunn et al. (1942). Thyroprotein treatment on vitamin A deficient rams appeared to have diminished the harmful effects of the vitamin A deficiency on total sperms per ejaculate. This has not been reported in the literature. The favorable

CONTROL —————
 A⁺ + PMS - - - - -
 A⁺ + THYROPROTEIN — x — x —
 A⁺ + TESTOSTERONE - . - . -

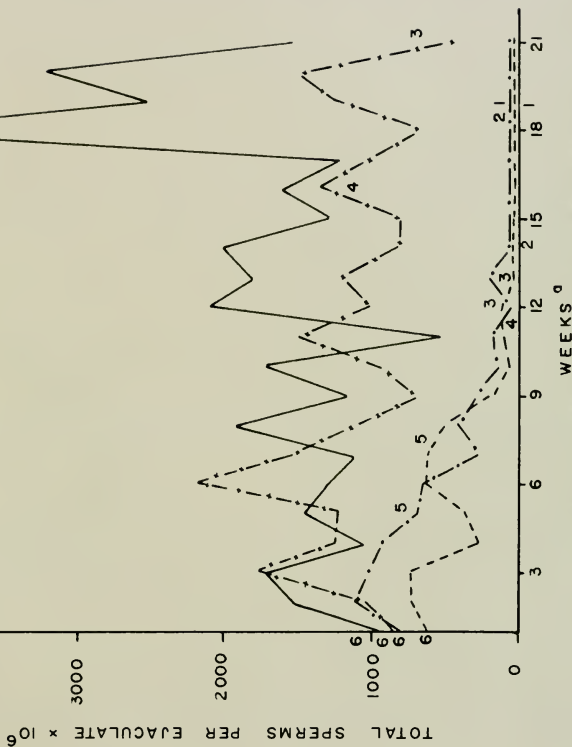


Figure 17.--Effect of various hormone treatments on total sperm production of vitamin A deficient rams.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. Differences in numbers between weeks in a given group resulted from death loss.

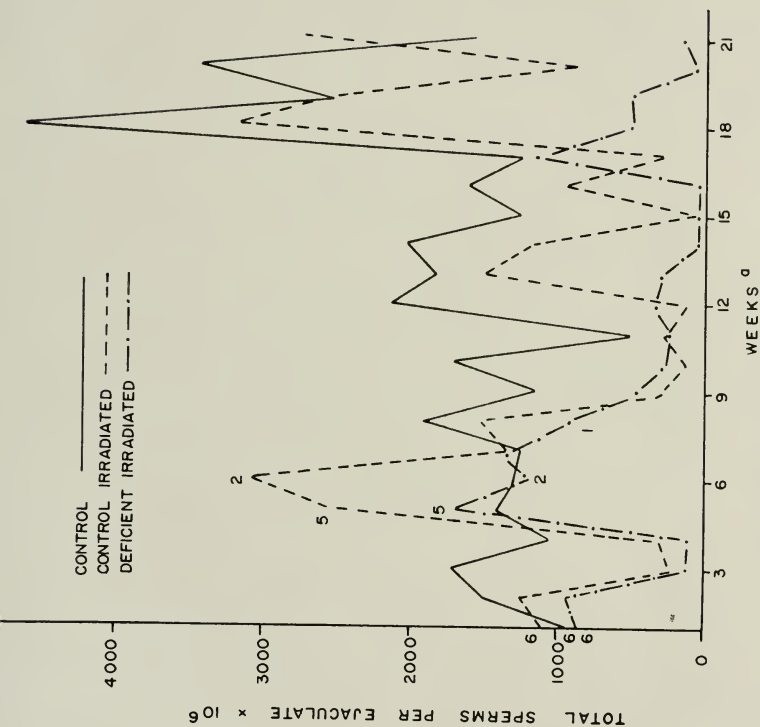


Figure 18.--Effect of irradiation on total sperm production of vitamin A deficient and normal rats.

^aNumbers adjacent to plotted values represent animals remaining in each group for a given week. Differences in numbers between weeks in a given group resulted from death loss.

effect of thyroprotein on sperm production is not easily explained since histological examination of the testes from all the vitamin A deficient rams showed a reduction in the germinal epithelium of the seminiferous tubules. It is possible that thyroprotein might have delayed the histological changes thus allowing continuation of spermatogenesis for a longer period. To confirm this however, a series of testicular biopsies would have been necessary to ascertain when the histological lesions began to occur in the vitamin A deficient rams. The effects of irradiation on sperm production can be explained if one assumes that irradiation affected spermatogenesis in the early stages of the process, so that when new spermatocytes were produced normal sperm production was resumed. This would explain the recovery in sperm production observed in the normal irradiated group 16 weeks after irradiation.

Individual data on total sperm per ejaculate throughout phase II are shown in the Appendix, Table 24. Analysis of variance² for differences in total sperm per ejaculate are shown in Tables 30 through 39 in the Appendix.

It is appropriate at this point to draw several conclusions on the effects of the various treatments on semen characteristics. Before doing so it is emphasized that experimental numbers were limited and large death losses made the number of observations even smaller. This and the variability in the data deprived the biological differences observed of statistical significance in most instances. Semen volume of vitamin A deficient rams was reduced. This effect was present before the external signs normally associated with a vitamin A deficiency were observed on the majority of the rams. This effect on semen volume was not prevented by treatment

with testosterone, PMS or thyroprotein. Sperm motility was severely affected by a vitamin A deficiency. This effect did not become evident until the nervous lesions associated with a vitamin A deficiency began to show in most of the experimental animals. PMS or testosterone treatment had no effect on preventing changes in sperm motility on the vitamin A deficient rams. Thyroprotein was of some benefit. Irradiation appeared to have had a detrimental effect on sperm motility values in both groups irradiated. An increase in number of abnormal sperm occurred in vitamin A deficient and irradiated rams. Hormones used had no beneficial effect in reducing the percentage of abnormal sperms present. Total sperm production was severely affected by the vitamin A deficient condition. Manifestation of this effect occurred in association with a decrease in semen volume. Of the hormones used only thyroprotein appeared to have had any beneficial effect on sperm production. Histological examination indicated that the reduced sperm production was due to a reduction in the germinal epithelium of the seminiferous tubules which caused an impairment in spermatogenesis. The ineffectiveness of PMS or testosterone on improving semen characteristics would suggest that the effects of a vitamin A deficiency on semen traits do not occur through a secondary effect from imbalances or reduced secretion of hormones normally associated with spermatogenesis.

Histological Investigation

Quantitative evaluation of testes of rams from the various treatments is shown in Table 14.

TABLE 14
QUANTITATIVE EVALUATION OF TESTES FROM RAMS
IN THE VARIOUS TREATMENTS

Treatment Group	No. of Samples	Tubule Epithelium Thickness (mm)	Tubule Diameter (mm)	Interstitial ^a Tissue
Control	5	.211	.718	2
Deficient	5	.054	.475	4.4
Returned	6	.207	.744	2.1
A ⁻ + PMS	5	.032	.491	3.0
A ⁻ + Thyroprotein	4	.094	.587	4.2
A ⁻ + Testosterone	4	.047	.452	3.8
A ⁻ Irradiated	4	.068	.614	4.5
Control Irradiated	4	.182	.683	2.1

^aInterstitial tissue scored from 1 to 5; 1, sparse, and 5 abundant.

Vitamin A deficiency resulted in a reduction in the germinal epithelium thickness in all rams on the vitamin A deficient diet. The diameter of the seminiferous tubules also appeared reduced. Similar lesions have been reported in rats by Mason (1933), Howell et al. (1963), in bulls, Bratton et al. (1948) and in rams by Lindley et al. (1949). None of the hormones used had any effect in preventing testicular damage.

Interstitial tissue was not affected by the vitamin A deficient condition. There was more interstitial tissue in rams on the deficient diet than in those on the diet containing vitamin A. The reduced amounts of interstitial tissue present in the testes of vitamin A supplemented rams can not be explained. Figures 19 and 20 illustrate the lack of epithelium thickness in the seminiferous tubules of a vitamin A deficient ram compared to a control. Note also the sparse interstitial tissue present in the testes of the control ram.

Thyroid tissues from the control, deficient and A⁻ + Thyroprotein rams were studied for differences in thickness of the follicular wall cells. Table 15 shows average epithelium thickness for the 3 treatments. Based on the small number of thyroid samples studied there appeared to be no differences in thyroid secretory tissue activity between the 3 treatments.

Histological studies made on the adenoypophysis of rams on the various treatments revealed no unusual degree of degranulation and vacuolation present. Even though the observations made of the adenoypophysis were limited in numbers, the lack of abnormal hypophyseal development suggests no alteration in physiological processes that were dependent upon the pituitary hormones due to the vitamin A deficiency in these rams.

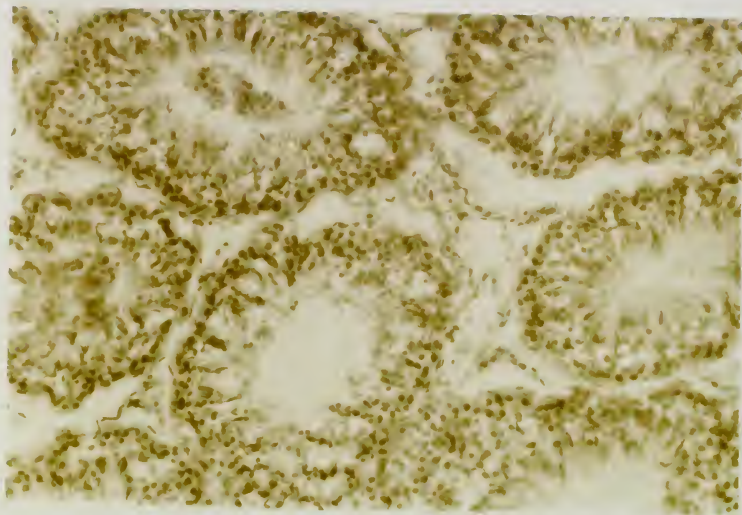


Figure 19.--Seminiferous tubules in testes from a control ram.

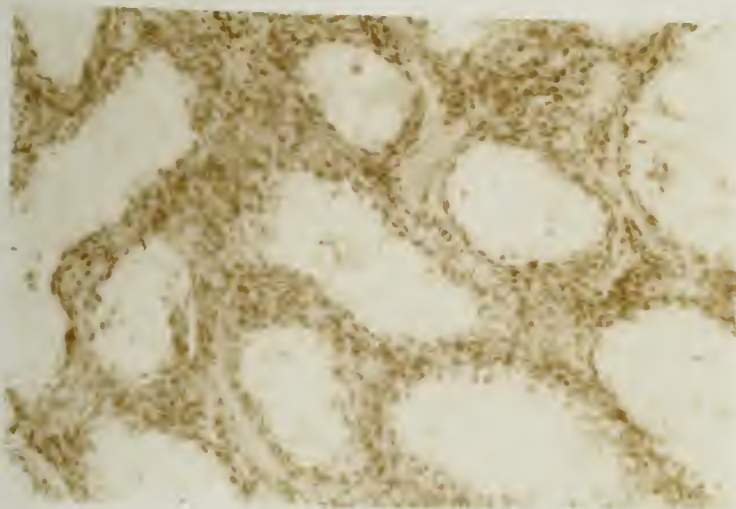


Figure 20.--Seminiferous tubules in testes from a vitamin A deficient ram.

No cystic pituitaries were found in any of the vitamin A deficient rams. This is contrary to the findings of Lindley et al. (1949); they reported cystic pituitaries in 3 to 4 month old rams exposed to a vitamin A deficient diet. It is possible that the difference in age and development at the time of vitamin A deprivation between the rams in the studies of Lindley et al. (1949) and those used in this study could account for differences in pituitary condition. However Erb et al. (1947) found cystic pituitaries in mature dairy bulls exposed to a vitamin A deficient diet over a prolonged period of time.

Individual measurements on testes and thyroid are shown in the Appendix Table 23.

TABLE 15

QUANTITATIVE EVALUATION OF THE THYROID FROM RAMS IN THE
CONTROL, DEFICIENT, AND A⁻ + THYROPROTEIN GROUPS

Treatment	No. of Samples	Epithelium Thickness (mm)
Control	5	.038
Deficient	3	.054
A ⁻ + Thyroprotein	5	.041 ⁷

Slaughter Data

Table 16 shows weights of the various glands taken at death of rams. Differences in gland weights due to treatment are shown in Table 17.

TABLE 16

SUMMARY OF GLAND WEIGHTS TAKEN AT TIME OF SLAUGHTER

Treatment	Gm. per 100 lbs. body weight					
	Testes	Epididymis	Seminal Vesicles	Cowpers	Adrenals	Thyroid Pituitary
Control	280.5	40.7	36.6	2.4	2.2	3.6 .634
Deficient	115.7	44.9	35.8	2.3	3.2	3.2 .709
Returned	268.5	48.3	38.4	2.8	2.0	3.9 .626
A ⁻ + PMS	104.5	38.6	31.3	2.6	3.2	4.5 .560
A ⁻ + Thyroprotein	143.4	33.1	29.1	2.9	2.8	3.6 .342
A ⁻ + Testosterone	81.7	35.0	32.5	4.8	5.5	4.2 .684
Control Irradiated	219.0	42.8	30.8	1.6	2.5	3.0 .545
A ⁻ Irradiated	186.9	47.6	22.0	1.6	4.3	6.6 .583

TABLE 17

DIFFERENCES IN GLAND WEIGHT BETWEEN THE VARIOUS TREATMENTS

Gland g./100 lbs. body weight		Ranked Means for the Various Treatments ^a							
Testes	Control	Returned	Control Irradiated	A ⁻ Irradiated	A ⁻ + Thyroprotein	Deficient	A ⁻ + PMS	A ⁻ + Testosterone	
	280.5	268.5	219.0	186.9	143.4	115.7	104.5	81.7	
Epididymids	Returned	A ⁻ Irradiated	Deficient	Control Irradiated	Control	A ⁻ + PMS	A ⁻ + Testosterone	A ⁻ + Thyroprotein	
	48.3	47.6	44.9	42.8	40.7	38.6	35.0	33.1	
Seminal Vesicles	Returned	Control	Deficient	A ⁻ + Testosterone	A ⁻ + PMS	Control Irradiated	A ⁻ + Thyroprotein	A ⁻ Irradiated	
	38.4	36.6	35.8	32.5	31.3	30.8	29.1	22	
Cowpers	A ⁻ + Testosterone	A ⁻ + Thyroprotein	Returned	A ⁻ + PMS	Control	Deficient	Control Irradiated	A ⁻ Irradiated	
	4.8	2.9	2.8	2.6	2.4	2.3	1.6	1.6	
Adrenals	A ⁻ + Testosterone	A ⁻ + Irradiated	A ⁻ + PMS	Deficient	A ⁻ + Thyroprotein	Control Irradiated	Control	Returned	
	5.5	4.3	3.2	3.2	2.8	2.5	2.2	2.0	

TABLE 17 (Continued)

Gland g./100 lbs. body weight	Ranked Means for the Various Treatments ^a							
	A ⁻ Irradiated	A ⁻ + PMS	A ⁻ + Testosterone	Returned	Thyroprotein	Control	Deficient	Control Irradiated
Thyroid	6.6	4.5	4.2	3.9	3.6	3.6	3.2	3.0
Pituitary	Deficient .709	A ⁻ + Testosterone .684	Control .634	Returned .626	A ⁻ Irradiated .583	A ⁻ + PMS .560	Control Irradiated .545	A ⁻ + Thyroprotein 342

^aMeans underscored by the same line are not significantly ($P < .05$) different.

Testes were significantly heavier in rams in the control and returned groups than in all other treatments. Rams in the A⁻ irradiated group had significantly heavier testes than other rams in the vitamin A deficient diet. This was probably due to the fact that most of the rams on this treatment died much earlier in the experiment because of irradiation exposure thus were subjected to vitamin A deprivation for a considerable shorter period of time. PMS or testosterone treatment did not prevent testicular atrophy in vitamin A deficient rams. Thyroprotein treated rams had slightly heavier testes than rams in the deficient group but this difference was not significant.

Results obtained in this experiment show that vitamin A deficiency causes a reduction in testicular size. This effect does not appear to be secondary to a testosterone deficiency since testosterone administration was not effective in preventing the reduction in testicular size.

The reduced testicular weights associated with vitamin A deficiency in rams have been reported by Lindley et al. (1949). Similar results were reported in rats by Gross (1924), Wolbach (1925), Mayer and Truant (1949) and Howell et al. (1963). Mayer and Truant (1949) by injecting testosterone were able to obviate testicular atrophy in vitamin A deficient rats, Lindley et al. (1949) were unsuccessful in preventing testicular atrophy in rams with injections of testosterone. The results of this experiment are in agreement with the findings of Lindley et al. (1949).

Epididymal weight comparison between the various treatment groups is rather inconclusive. Rams in the deficient, A⁻ + PMS and A⁻ + Testosterone groups did not show significantly different epididymal size from

the control rams. Rams on the A⁻ + Thyroprotein groups showed significantly smaller epididymal size than deficient and control rams.

There were no significant differences in seminal vesicle weight between rams in the different treatments.

The cowpers of rams in the A⁻ + Testosterone were significantly heavier than those of rams in all other treatments. Rams in both irradiated treatments had significantly smaller cowpers than rams in all other treatments. Vitamin A deficiency appeared to have had no effect on cowpers development in rams. The explanation for this irradiation effect is not known. Testosterone treatment had a stimulating effect on the development of the cowpers. This is to be expected since it has long been known that the functioning of the cowpers is under testosterone influence (Turner, 1961).

Statistical differences in adrenal size between the various treatments are not conclusive. However in general rams in the control and returned groups showed smaller adrenals.

A significantly heavier thyroid was present in rams in the A⁻ irradiated group. However rams in the control irradiated group had the smallest average thyroid size. So that if irradiation per se had an effect on thyroid development this was only evident in vitamin A deficient rams. No other effects on thyroid development were observed.

Pituitary weight did not appear to be affected by the vitamin A status of rams. Treatment with either testosterone or PMS did not appear to have any definite effect on pituitary development. Had the level of thyroprotein given been sufficient for normal body functioning, pituitary

thyrotrophic hormone secretion would have been reduced. Consequently a reduction in thyroid size from lack of thyrotrophic hormone stimulation could have resulted. It is interesting to note that thyroprotein treatment did not cause a reduction in thyroid size. However the data are not conclusive enough to permit a definite statement on this relationship.

Table 25 in the Appendix show individual gland size for all rams. The reduced number of rams and the variability in gland size between rams within a treatment suggest caution in making any categoric statements on hormonal and nutritional interrelationships in these rams.

Analysis of variance for differences in testes, epididymis, seminal vesicles, cowpers, adrenals thyroid and pituitary size are shown in the Appendix Tables 40, 41, 42, 43, 44, 45 and 46 respectively.

SUMMARY

A study was conducted to determine the effects of a vitamin A deficiency on the reproductive capacity of rams. An attempt was made to counteract the harmful effects of the vitamin A deficiency by administering testosterone, pregnant mare serum (PMS) or thyroprotein. Information was obtained on the tolerance of vitamin A deprived rams to gamma irradiation.

The experiment was started on February 2, 1963. During phase I of the experiment 12 yearling rams were assigned as controls to a purified diet containing 3,000 I.U. of vitamin A per lb. of feed. Thirty-six yearling rams were placed on the vitamin A free diet. During phase II the 12 rams on the control diet with average vitamin A values of 139 mcg./100 ml. plasma and 156 mcg./g. fresh liver were continued on the same ration with 6 serving as controls and 6 irradiated with 400 r of whole body gamma irradiation. During phase II the 36 rams on the vitamin A free diet with vitamin A values averaging 5 mcg./100 ml. plasma and 5.6 mcg./g. fresh liver were continued on the vitamin A free diet and were assigned in equal groups to one of 6 treatments: (1) 400 r of whole body gamma irradiation; (2) Daily injections of 60 RU of PMS/ 100 lbs. body weight; (3) Daily injections of 10 mg. of testosterone/100 lbs. body weight; (4) Daily capsules of 1 g. thyroprotein/100 lbs. body weight; (5) 3000 I.U. of vitamin A/lb. of feed; (6) No additional treatment. Semen was checked monthly during phase I and weekly for 21 weeks during phase II.

Night blindness, cloudiness of the cornea, uncoordinated gait and extreme weakness were symptoms associated with rams on the vitamin A free

diet. Limited internal hemorrhaging in the chest cavity was characteristic of rams exposed to irradiation. Rams on the vitamin A deficient diet consumed less feed and lost more weight than controls. During phase II PMS or testosterone treatment did not have any beneficial effect on appetite or growth of vitamin A deficient rams. The thyroprotein treated rams consumed more feed and lost less weight than other rams on the vitamin A deficient diet. Irradiation appeared to have had a depressing effect on feed intake and growth of all animals irradiated. Rams whose vitamin A reserves had been drastically reduced and were experiencing severe weight losses were able to recover and suffered no death losses when 3,000 I.U. of vitamin A per lb. of feed were provided. Most of the rams whose vitamin A reserves had been depleted eventually died. Treatment with either testosterone or PMS was not effective in preventing death losses in deficient rams. Thyroprotein capsules did reduce death loss of deficient rams. The vitamin A status of rams exposed to irradiation did not affect their survival time. Vitamin A deficiency caused a reduction of germinal epithelium thickness and in the diameter of the seminiferous tubules of testes. Histological studies of the thyroid and adenohypophysis revealed no changes that could be attributed to vitamin A status of rams. Semen volume, sperm motility, per cent abnormal sperm and total sperm production were severely affected by vitamin A deficiency. Sperm motility and total sperm production changes were ameliorated with thyroprotein treatment. Irreparable damage of semen traits studied did not result from a drastic reduction of vitamin A reserves. Irradiation appeared to increase per cent of abnormal sperm cells and reduced motility values in rams with normal vitamin A

reserves. Testicular weight was reduced in all vitamin A deficient rams. Epididymis cowper's, seminal vesicles, adrenals, thyroid and pituitary weights were not affected by the vitamin A deficiency.

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APPENDIX

TABLE 18
INITIAL^a AND FINAL VITAMIN A PLASMA AND LIVER VALUES^b FOR
INDIVIDUAL RAMS

Treatment Group and Animal No.	Initial		Final
	mcg./100 ml. plasma	mcg./g. fresh liver	mcg./100 ml. plasma
Control			
13	21	130	54
15	45	162	21
Y97	54	228	60
22	33	154	34
G9	54	127	27
Y43	30	23	15
Deficient			
Y19	0	.6	9
B9	9.0	1.2	- ^c
B32	1.5	0	- ^c
B83	1.2	2.0	- ^c
Y44	6.0	3.7	- ^c
Returned			
G99	0	.6	42
G94	3.0	1.5	30
Y47	7.5	1.4	40
B2	1.5	0	35
Y22	12	69.6	24
Y99	27	34.6	30

TABLE 18 (Continued)

Treatment Group and Animal No.	Initial		Final
	mcg./100 ml. plasma	mcg./g. fresh liver	mcg./100 ml. plasma
A ⁻ + Thyroprotein			
Y16	3.0	.6	9.0
Y24	6.0	1.5	3.0
Y83	0	1.1	- ^c
Y98	4.5	0	- ^c
G26	6.0	4.3	- ^c
Y75	3.0	3.8	- ^c
A ⁻ + PMS			
B21	0	.9	
3	0	4.2	
Y81	9	2.9	- ^c
Y20	18	1.7	- ^c
Y84	1.9	1.9	- ^c
Y50	0	0	0
A ⁻ + Testosterone			
Y80	6.0	.6	6.0
B19	0	1.2	0
Y100	4.5	1.4	- ^c
Y95	1.5	2.0	- ^c
G87	1.2	4.5	- ^c
Y46	3.0	4.6	- ^c

TABLE 18 (Continued)

Treatment Group and Animal No.	Initial		Final
	mcg./100 ml. plasma	mcg./g. fresh liver	mcg./100 ml. plasma
Control Irradiated			
G90	12	118	51
G97	21	276	- ^c
6	42	111	- ^c
21	60	125	- ^c
Y79	63	193	39
16	33	124	- ^c
A ⁻ Irradiated			
G1	3.8	.3	- ^c
G92	6.0	6.3	3
Y74	12.0	12.3	- ^c
Y85	3.0	5.8	- ^c
B52	19.0	19.0	- ^c
37	2.6	2.6	- ^c

^aValues obtained at time of reassignment.

^bFinal liver values were not determined.

^cAnimal died prior to termination of experiment.

TABLE 19

Treatment Group and Animal No.	Phase I	Phase II	Combined
Control			
13	.26	.12	.17
15	.18	.13	.15
Y97	.22	.04	.11
22	.30	.05	.15
G9	.21	-.01	.11
Y43	.27	-.33	-.02
Av.	.24	0	.11
Deficient			
Y19	-.18	.01	-.06
B9	.24	-.23	-.02
B32	.05	-.12	-.01
B83	.11	-	* .11
Y44	.15	-.18	.04
Av.	.06	-.10	-.04
Returned			
G99	.16	.26	.22
G94	.13	.10	.11
Y47	.16	.10	.09
B2	-.11	.28	.12
Y22	.21	-.16	.03
Y99	.07	.09	.08
Av.	.10	.11	.11

TABLE 19 (Continued)

Treatment Group and Animal No.	Phase I	Phase II	Combined
A^- + Thyroprotein			
Y76	-.08	.11	.04
Y24	.16	.03	.08
Y83	.04	-.02	.02
Y98	.10	-.12	-.04
G26	.14	-.14	-.01
Y75	-.01	-.12	-.05
Av.	.06	-.04	.01
A^- + PMS			
B21	-.18	-.02	-.09
3	.02	.21	-.04
Y81	.16	-.08	.02
Y20	.09	-.17	-.06
Y84	.16	-.08	.02
Y50	.06	.12	-.04
Av.	.05	-.04	-.03
A^- + Testosterone			
Y80	-.09	-.09	-.09
B19	.13	-.09	0
Y100	.03	-.31	-.12
Y95	.02	-.39	-.06
G87	.19	-.44	0
Y46	-.06	-.31	-.16
Av.	.04	-.27	-.07

TABLE 19 (Continued)

Treatment Group and Animal No.	Phase I	Phase II	Combined
Control Irradiated			
G90	.15	.15	.15
G97	.32	-.54	.14
6	.23	-.39	.11
21	.42	-.46	.25
Y79	.14	-.01	.24
16	.10	-.11	.7
Av.	.23	-.23	.16
A ⁻ Irradiated			
G1	.02	-.07	.01
G92	-.02	-.11	-.08
Y74	.09	-.32	.01
Y85	.14	-.14	.07
B52	-.02	-.43	-.08
37	.14	-.33	.04
Av.	.06	-.23	-.01

TABLE 20

SUMMARY OF THE 14-DAY PERIODS WEIGHT OF INDIVIDUAL
RAMS AND TREATMENT GROUPS IN PHASE I (POUNDS)

Treatment Group and Animal No.	Periods										
	1	2	3	4	5	6	7	8	9	10	11
Control											
13	91	101	101	100	111	113	-	-	-	-	-
15	102	107	113	116	116	121	-	-	-	-	-
Y97	113	116	118	116	124	129	130	134	-	-	-
22	76	77	85	87	94	100	103	107	-	-	-
G9	76	79	88	83	90	93	101	100	108	109	115
Y43	95	90	98	104	112	122	125	134	136	140	136
Deficient											
Y19	78	71	68	64	65	66	-	-	-	-	-
B9	83	84	94	95	96	100	99	103	-	-	-
B32	67	63	70	68	72	70	70	72	-	-	-
B83	81	83	85	94	100	103	99	104	105	104	101
Y44	83	83	87	93	100	108	114	116	119	106	118
Returned											
G99	77	74	83	86	89	94	-	-	-	-	-
G94	76	77	79	79	83	89	-	-	-	-	-
Y47	87	88	98	98	102	103	105	105	-	-	-
B2	75	71	80	76	67	69	65	65	-	-	-
Y22	89	85	90	97	107	107	110	114	117	120	123
Y99	85	90	96	100	104	111	114	113	115	112	109

TABLE 20 (Continued)

Treatment Group and Animal No.	Periods										
	1	2	3	4	5	6	7	8	9	10	11
A⁻ + Thyroprotein											
Y76	97	88	93	93	95	99	-	-	-	-	-
Y24	90	89	96	97	101	105	-	-	-	-	-
Y83	87	84	92	94	90	91	91	95	-	-	-
Y98	81	79	78	83	90	91	92	94	-	-	-
G26	79	78	88	96	104	107	105	106	105	108	108
Y75	98	96	102	102	104	105	105	105	105	105	108
A⁻ + PMS											
B21	81	81	77	72	74	71	-	-	-	-	-
3	104	100	106	104	106	112	-	-	-	-	-
Y81	95	95	100	103	107	114	118	117	-	-	-
Y20	94	85	86	86	91	95	95	98	-	-	-
Y84	74	77	84	84	92	92	89	96	98	99	99
Y50	91	88	93	85	86	91	91	96	99	103	105
A⁻ + Testosterone											
Y80	78	79	79	75	74	76	-	-	-	-	-
B19	84	84	90	88	95	95	-	-	-	-	-
Y100	78	78	84	73	76	87	85	83	-	-	-
Y95	83	83	84	78	88	91	81	87	-	-	-
G87	78	78	78	81	91	95	102	107	106	107	110
Y46	112	112	114	114	118	116	120	127	118	118	118

TABLE 20 (Continued)

Treatment Group and Animal No.	Periods										
	1	2	3	4	5	6	7	8	9	10	11
Control Irradiated											
G90	82	84	90	90	95	101	-	-	-	-	-
G97	102	96	105	111	125	130	-	-	-	-	-
6	79	77	88	87	96	100	103	102	-	-	-
21	94	96	107	108	107	118	125	130	-	-	-
Y79	75	77	81	79	86	89	90	90	95	100	99
16	91	84	86	84	89	96	103	100	105	106	107
A ⁻ Irradiated											
G1	95	98	102	100	104	102	-	-	-	-	-
G92	94	85	89	90	96	99	-	-	-	-	-
Y74	85	86	87	91	91	95	95	91	-	-	-
Y85	90	88	94	92	100	104	103	104	-	-	-
B52	86	86	93	92	98	100	96	95	91	87	85
37	95	97	101	109	112	112	113	113	114	108	118

TABLE 21

SUMMARY OF THE 14-DAY PERIODS WEIGHT OF INDIVIDUAL
RAMS AND TREATMENT GROUPS IN PHASE II (POUNDS)

Treatment Group and Animal No.	Periods										
	1	2	3	4	5	6	7	8	9	10	11
Control											
13	113	120	121	122	127	136	129	134	130	130	128
15	117	128	134	133	139	140	137	136	132	140	138
Y97	138	130	135	139	139	140	147	142	144	145	148
22	110	106	112	116	118	122	117	118	118	106	120
G9	110	106	115	115	119	117	116	118	117	110	108
Y43	137	140	135	124	126	120	112	107	107	102	92
Deficient											
Y19	63	70	69	70	76	73	73	71	69	67	65
B9	110	102	98	98	97	96	94	94	90	82	78
B32	73	73	72	68	-	-	-	-	-	-	-
B83	98	-	-	-	-	-	-	-	-	-	-
Y44	102	110	109	106	102	96	92	- ^a	-	-	-
Returned											
G99	91	96	104	111	108	118	115	122	123	126	129
G94	87	90	94	100	96	104	95	100	107	101	102
Y47	105	101	103	110	109	113	111	114	104	115	112
B2	63	63	62	69	66	81	97	94	101	105	107
Y22	122	116	118	115	114	112	111	110	110	107	108
Y99	96	112	108	105	102	102	103	105	104	102	100

TABLE 21 (Continued)

Treatment Group and Animal No.	Periods										
	1	2	3	4	5	6	7	8	9	10	11
A⁻ + Thyroprotein											
Y76	90	96	99	100	96	101	103	113	113	118	114
Y24	104	111	113	121	118	120	122	122	122	115	108
Y83	91	86	82	85	87	98	97	101	98	90	92
Y98	92	89	91	93	95	94	91	90 ^a	84	83	72
G26	100	103	100	91	89	85	80	77	- ^a	-	-
Y75	97	109	108	100	94	88	85	76	- ^a	-	-
A⁻ + PMS											
B21	69	69	61	64	68	- ^a	-	-	-	-	-
3	106	109	100	- ^a	-	-	-	-	-	-	-
Y81	113	111	113	118	109	110	109	106	102	101	- ^a
Y20	104	101	90	103	91	98	87	82	- ^a	-	-
Y84	99	96	92	92	90	78	- ^a	-	-	-	-
Y50	100	96	97	93	91	81	81	- ^a	-	-	-
A⁻ + Testosterone											
Y80	70	72	72	71	68	77	66	68	65	64	58
B19	95	102	102	92	98	106	101	101	92	83	- ^a
Y100	85	86	84	79	79	68	59	- ^a	-	-	-
Y95	81	73	70	- ^a	-	-	-	-	-	-	-
G87	110	109	102	95	90	79	- ^a	-	-	-	-
Y46	106	114	102	95	92	91	84	83	71	- ^a	-

TABLE 21 (Continued)

Treatment Group and Animal No.	Periods										
	1	2	3	4	5	6	7	8	9	10	11
Control Irradiated											
G90	95	86	83	84	86	86	93	104	109	116	110
G97	129	123	114	- ^a	-	-	-	-	-	-	-
6	105	101	94	- ^a	-	-	-	-	-	-	-
21	141	135	128	- ^a	-	-	-	-	-	-	-
Y79	96	87	90	85	91	86	90	92	94	95	96
16	107	99	104	- ^a	-	-	-	-	-	-	-
A ⁺ Irradiated											
G1	97	96	- ^a	-	-	-	-	-	-	-	-
G92	92	92	90	84	82	85	86	79	84	80	81
Y74	95	93	86	- ^a	-	-	-	-	-	-	-
Y85	106	105	100	- ^a	-	-	-	-	-	-	-
B52	83	73	71	- ^a	-	-	-	-	-	-	-
31	116	111	110	102	- ^a	-	-	-	-	-	-

^aAnimal died.

TABLE 22
SURVIVAL TIME^a FOR INDIVIDUAL RAMS

Treatment Group and Animal No.	Days Survived
Control	
13 -----	141
15 -----	141
Y97 -----	141
22 -----	141
G9 -----	141
Y43 -----	141
Av. -----	141
Deficient	
Y19 -----	141
B9 -----	136
B32 -----	44
B83 -----	1
Y44 -----	88
Av. -----	44
Returned	
G99 -----	141
G94 -----	141
Y47 -----	141
B2 -----	141
Y22 -----	141
Y99 -----	141
Av. -----	141

TABLE 22 (Continued)

Treatment Group and Animal No.	Days Survived
A^- + Thyroprotein	
Y76 -----	141
724 -----	141
Y83 -----	141
Y98 -----	133
G26 -----	95
Y75 -----	98
Av. -----	96
A^- + PMS	
B21 -----	66
3 -----	38
Y81 -----	120
Y20 -----	83
Y84 -----	64
Y50 -----	141
Av. -----	102 ₂
A^- + Testosterone	
Y80 -----	141
B19 -----	124
Y100 -----	81
Y95 -----	21
G87 -----	72
Y46 -----	141
Av. -----	106

TABLE 22 (Continued)

Treatment Group and Animal No.	Days Survived
Control Irradiated	
G90 -----	141
G97 -----	28
6 -----	25
21 -----	24
Y79 -----	141
16 -----	20
Av. -----	80
A ⁻ Irradiated	
G1 -----	25
G92 -----	141
Y74 -----	21
Y85 -----	24
B52 -----	25
37 -----	37
Av. -----	36

^aFrom reassignment to death resulting from treatment or slaughtering after 141 days.

TABLE 23
QUANTITATIVE EVALUATION OF TESTES AND THYROID

Treatment Group and Animal No.	Testes			Thyroid
	Epithelium Thickness (mm.)	Tubule Diameter (mm.)	Interstitial Tissue ^a	Epithelium Thickness (mm.)
Control				
13	.297	.837	2	.014
15	.189	.729	2	.014
Y97	.135	.540	4	- ^a
22	.243	.864	1	.027
G9	.189	.621	1	.054
Y43	- ^b	- ^b	- ^b	.081
Av.	.211	.718	2	.038
Deficient				
Y55	.054	.486	5	.027
Y44	.0	.486	5	.054
Y19	.189	.513	2	.081
B83	.027	.513	5	- ^b
B32	.0	.378	5	- ^b
Av.	.054	.475	4.4	.054
Returned ^c				
Y22	.189	.756	5	
YD47	.216	.681	1	
G94	.216	.648	2	
B2	.135	.783	1	

TABLE 23 (Continued)

Treatment Group and Animal No.	Epithelium Thickness (mm.)	Testes		Thyroid Epithelium Thickness (mm.)
		Tubule Diameter (mm.)	Interstitial Tissue ^a (mm.)	
G99	.324	.837	3	
Y99	.162	.756	1	
Av.	.207	.744	2.1	
A ⁻ + Thyroprotein				
Y083	.053	.459	3	.054
Y24	.0	.486	5	.027
Y75	.081	.594	5	.054
Y76	.243	.810	4	.014
Y98	_b	_b	_b	.054
G26	_d	_d	_d	_d
Av.	.094	.587	4.2	.041
A ⁻ + PMS				
Y20	.081	.486	5	
Y50	.0	.486	1	
Y81	.027	.486	5	
Y84	.054	.540	2	
3	0	.459	2	
B21	_b	_b	_b	
Av.	.032	.491	3	
A ⁻ + Testosterone ^c				
Y100	.054	.513	5	
Y080	.027	.351	4	

TABLE 23 (Continued)

Treatment Group and Animal No.	Testes			Thyroid
	Epithelium Thickness (mm.)	Tubule Diameter (mm.)	Interstitial Tissue ^a (mm.)	Epithelium Thickness (mm.)
G87	.027	.459	3	#
Y46	.081	.486	3	
B19	_b	_b	_b	
Y95	_b	_b	_b	
Av.	.047	.452	3.8	
Control Irradiated				
690	.243	.783	2	
G97	.135	.681	5	
6	.216	.540	2	
21	_d	_d	_d	
Y79	.135	.729	0	
16	_d	_d	_d	
Av.	.182	.683	2.1	
A ⁻ Irradiated				
G1	0	.540	5	
G92	_d	_d	_d	
Y85	.081	.810	5	
B52	.135	.648	3	
37	_d	_d	_d	
Y74	.054	.459	5	
Av.	.068	.614	4.5	

^aInterstitial tissue scored from 1 to 5; 1, sparse, and 5, abundant.

^bSample lost in processing.

^cThyroid tissue was not evaluated in animals on this treatment.

^dAnimals found in a decomposing state.

TABLE 24

SUMMARY OF THE SEMEN VOLUME, MOTILITY, ABNORMAL CELLS AND THE TOTAL SPERM CELL PRODUCTION FOR INDIVIDUAL RAMS AND TREATMENT GROUPS BY WEEKS

Treatment Group and Animal No.	1				2				3			
	Vol. ^a	Mot. ^b	Abn. ^c	TCPd	Vol. ^a	Mot. ^b	Abn. ^c	TCPd	Vol. ^a	Mot. ^b	Abn. ^c	TCPd
Control												
13	1.2	55	46	868	1.5	64	17	1427	1.7	83	42	4264
15	.9	58	51	540	.9	73	52	1224	.9	53	76	2041
Y97	1.2	86	8	1652	1.3	85	0	955	1.1	90	6	1311
22	.7	85	13	667	1.2	85	2	3274	1.5	80	25	765
G9	.7	83	30	942	.5	75	0	1036	.8	68	0	499
Y43	1.1	76	27	758	1.0	90	3	1441	1.7	85	4	1387
Deficient												
Y19	.5	39	75	275	.5	16	85	478	1.4	8	66	2120
B9	.8	63	23	792	.5	76	19	548	.6	85	22	1093
B32	.9	30	57	487	.5	6	63	328	.8	10	33	620
B83	.7	0	77	715	^e	-	-	-	-	-	-	-
Y44	.6	60	26	367	.5	59	13	115	.5	68	17	177

TABLE 24 (Continued)

Treatment Group and Animal No.	1			2			3					
	Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd
Returned												
G99	.7	59	57	383	.6	76	49	1242	1.1	83	49	4223
G94	.6	72	35	415	1.3	64	25	1057	.9	43	34	1501
Y47	.6	78	12	875	.6	66	49	1111	.9	90	19	2136
B2	.4	0	37	72	.3	0	- ^f	0	.2	3	- ^f	0
Y22	.5	67	27	337	.4	60	6	209	1.2	70	10	1968
Y99	.8	60	32	555	.9	66	16	1340	1.1	80	6	1010
A ⁺ + Thyroprotein												
Y76	.9	84	52	2505	1.4	75	73	2499	2.4	50	71	5213
Y24	.7	60	55	503	1.1	70	27	380	.9	70	41	2057
Y83	.4	61	19	485	.7	74	44	1103	.7	18	63	439
Y98	.7	70	12	660	.5	64	36	388	1.1	15	72	984
G26	.7	80	50	459	.6	84	3 _u	894	.9	93	23	1230
Y75	.7	59	32	547	.6	75	36	1132	.7	78	8	578

TABLE 24 (Continued)

Treatment Group and Animal No.	1			2			3		
	Vol. ^a	Mot. ^b	Abn. ^c TCPd	Vol. ^a	Mot. ^b	Abn. ^c TCPd	Vol. ^a	Mot. ^b	Abn. ^c TCPd
A⁻ + PMS									
B21	.5	0	83	62	.2	0	- ^f	0	- ^f 0
3	.6	58	93	789	.6	36	57	695	.5 75 55 1452
Y81	.6	75	22	622	.4	61	40	463	.6 30 46 641
Y20	.6	65	44	226	.7	48	20	101	1.1 65 10 564
Y84	.9	89	30	901	.8	90	5	1309	.7 70 7 875
Y50	.7	69	47	1022	.8	35	44	2151	.7 00 14 831
A⁻ + Testosterone									
Y80	.4	60	59	397	.7	29	67	1475	.6 13 62 292
B19	1.1	83	60	917	1.2	71	76	817	1.3 70 57 1148
Y100	.6	66	8	1194	.5	84	38	2283	.8 78 60 1744
Y95	.7	15	32	587	.9	5	60	177	.7 10 34 63
G87	.7	62	24	646	.7	85	4	669	1.1 83 0 530
Y46	.9	78	29	1093	.7	50	19	850	.8 43 58 2416

TABLE 24 (Continued)

Treatment Group and Animal No.	1					2					3							
	Vol.	a	Mot.	b	Abn. c	TCPd	Vol.	a	Mot.	b	Abn. c	TCPd	Vol.	a	Mot.	b	Abn. c	TCPd
Control Irradiated																		
G90	.8		77	58	1403		.9		66	45	1008		1.7		45	55	4955	
G97	.9		68	55	550		1.4		81	12	1260		.8		35	25	2153	
6	.8		90	10	1584		.7		90	6	1401		1.1		65	13	1480	
21	.9		56	14	1253		.8		64	0	160		.5		53	5	228	
Y79	.9		86	32	1066		.7		54	13	831		.8		85	16	1244	
16	1.3		83	21	602		.9		88	6	2858		1.8		90	6	3367	
A ⁻ Irradiated																		
G1	.5		53	62	595		.6		50	92	930		.7		8	46	693	
G92	.5		72	23	464		.7		71	32	524		.5		55	31	1166	
Y74	1.3		52	8	1114		.7		88	9	967		3.1		80	20	2557	
Y85	1.0		81	23	1296		.6		94	0	1391		1.1		93	11	2611	
B52	.8		75	38	718		1.8		71	11	625		2.6		5	8	629	
37	7		74	33	960		.6		95	19	1114		.6		85	6	740	

TABLE 24 (Continued)

Treatment Group and Animal No.	4				5				6					
	Vol.	a	Mot.	b	Abn.	c	TCPd	Vol.	a	Mot.	b	Abn.	c	TCPd
Control														
13	1.6		88		6		2750	3.0		80		26		4308
15	1.0		40		20		902	.8		20		34		1344
Y97	.9		80		3		506	1.5		95		0		120
22	1.5		80		25		765	.8		90		15		1512
G9	.7		75		2		123	1.5		90		0		795
Y43	1.4		83		10		1215	.5		85		12		542
Deficient														
Y19	2.5		8		56		1060	2.0		55		84		250
B9	.7		40		38		1130	1.0		80		0		806
B32	.9		63		18		64	.5		40		38		20
Y44	.5		18		14		290	.5		5		48		605
												5		85
												55		32
												5		878
												10		68
												5		790
												31		735
												85		686

Deficient

TABLE 24 (Continued)

Treatment Group and Animal No.	4				5				6			
	Vol. ^a	Mot. ^b	Abn. ^c	TCP ^d	Vol. ^a	Mot. ^b	Abn. ^c	TCP ^d	Vol. ^a	Mot. ^b	Abn. ^c	TCP ^d
Returned												
G99	.9	70	47	1235	1.2	85	31	2050	1.0	80	44	907
G94	.9	38	27	562	1.2	50	31	1272	.8	80	3	1578
Y47	1.2	93	18	1919	1.9	90	9	2956	.9	90	12	771
B2	.5	0	-f	0	.3	60	-f	0	.2	0	-f	0
Y22	.6	30	24	296	.4	80	0	388	.6	80	13	93
Y99	1.4	88	22	1682	1.1	90	27	1663	.8	65	25	1290
A ⁻ + Thyroprotein												
Y76	2.3	23	68	4387	2.0	5	58	4680	1.8	70	44	3402
Y24	1.2	50	56	1624	1.1	70	52	1440	1.0	75	7	1460
Y83	.7 _h	3	45	198	.5	5	0	5	1.1	5	-f	0
Y98	.7	50	37	259	.5	30	50	255	1.4	75	13	2400
G26	.7	88	33	905	.8	80	0	232	1.4	95	45	2822
Y75	.9	60	4	175	.7	90	46	917	1.5	90	10	3062

TABLE 24 (Continued)

Treatment Group and Animal No.	4				5				6			
	Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd
A ⁻ + PMS												
B21	.4	0	-f	0	.3	0	-f	0	.5	0	-f	0
3	1.0	53	42	803	.8	20	27	624	1.8	20	49	2480
Y81	.6	30	46	193	.5	20	85	135	.6	20	45	6
Y20	.7	18	58	49	.5	0	-f	0	.8	90	-f	0
Y84	1.0	65	13	724	.8	80	31	1290	1.1	95	0	1192
Y50	.6	3	19	218	.5	0	52	233	.6	0	0	39
A ⁻ + Testosterone												
Y80	.6	0	-f	00	.7	0	-f	0	.9	0	-f	00
B19	1.5	40	38	2903	2.0	50	21	1700	1.6	80	6	1152
Y100	.6	8	77	444	.5	0	67	303	1.0	0	-f	0
Y95	.2	3	57	19	-.e	-	-	-	-	-	-	-
G87	.6	88	3	1325	.7	90	9	637	1.2	90	9	2026
Y46	.6	8	32	682	.8	0	2	1048	.6	0	67	165

TABLE 24 (Continued)

Treatment Group and Animal No.													
1				2				3					
Vol.	a	Mot.	b	Abn.	c	TCpd	Vol.	a	Mot.	b	Abn.	c	TCpd
Control Irradiated													
G90	2.8	20	71	8323	2.8	10	62	7056	1.6	20q	76	4272	
G97	1.2	65	9	1770	1.1	50	16	374	^e	-	-	-	
6	.7	25	28	375	2.0	90	1	1462	^e	-	-	-	
21	1.8	30	24	981	1.2	60	0	1906	^e	-	-	-	
Y79	1.1	90	30	2505	.6	85	69	1966	.7	80	65	1975	
16	3.0	80	10	4686	^e	-	-	-	-	-	-	-	
A ⁺ Irradiated													
G1	.8	5	59	430	1.0	5	63	545	^e	-	-	-	
G92	1.7	48	21	1306	2.2	20	45	4440	1.3	80	24	2158	
Y74	1.8	90	68	2843	^e	-	-	-	-	-	-	-	
Y85	7	80	37	229	1.8	80	17	1588	^e	-	-	-	
B52	2.5	48	42	982	.7	50	0	60	^e	-	-	-	
37	.9	90	4	1565	1.5	90	17	2004	.9	50	47	202	

A⁺ Irradiated

TABLE 24 (Continued)

Treatment Group and Animal No.	7				8				9			
	Vol. ^a	Mot. ^b	Abn. ^c	TCPd	Vol. ^a	Mot. ^b	Abn. ^c	TCPd	Vol. ^a	Mot. ^b	Abn. ^c	TCPd
Control												
13	1.0	90	24	2872	1.8	93	0	3579	1.2	88	13	1814
15	.7	80	47	1005	1.0	80	17	1895	1.3	48	23	3130
Y97	1.9	90	55	105	1.1	45	7	83	.9	75	2	217
22	1.5	90	8	2586	.8	70	15	430	.7	80	7	499
G9	.9	40	2	224	1.3	88	14	2605	.7	93	8	131
Y43	.8	80	6	196	1.4	95	26	2840	1.0	90	49	1263
Deficient												
Y19	1.0	5	62	275	1.2	8	61	331	.8	8	69	304
B9	.5	80	78	466	.5	8	9	140	.8	8	33	215
B32	- ^e	-	-	-	-	-	-	-	-	-	-	-
Y44	.6	20	70	354	.6	20	76	741	.5	0	17	222
Returned												
G99	.6	30	5	1104	1.2	85	4	2430	1.2	90	19	1578
G94	.8	90	13	1209	1.2	85	5	3323	.6	68	14	546

TABLE 24 (Continued)

Treatment Group and Animal No.	7				8				9					
	Vol.	a	Mot.	b	Abn.	c	TCPd	Vol.	a	Mot.	b	Abn.	c	TCPd
Y47	.8	90	0	846				.7	78	0	734	.9	93	8 1100
B2	.2	0	f	0				.3	0	f	0	.4	0	f 0
Y22	.5	40	16	275				.5	50	17	242	.5	80	12 141
Y99	1.3	90	26	1901				1.9	15	24	1796	.9	85	29 930
A ⁻ + Thyroprotein														
Y76	1.0	70	22	2293				.8	50	26	273	.6	45	20 128
Y24	.6	60	4	1345				1.0	88	8	1912	.7	93	13 1021
Y83	1.0	0	f	0				2.8	0	f	0	1.0	0	f 0
Y98	.4	40	0	64				.5	40	35	164	.4	65	11 210
G26	1.1	90	24	2440				.7	23	36	2073	.7	93	20 2429
Y75	1.2	60	32	3386				1.3	80	40	2389	1.4	0	30 869
A ⁻ + PMS														
B21	.2	0	f	0				.2	0	f	0	.1	0	f 0
3	.6	10	59	514				e	-	-	-	-	-	-
Y81	.4	10	9	152				.6	60	13	119	.4	15	8 36
Y20	.7	0	f	0				.9	8	30	178	.7	0	f 0

TABLE 24 (Continued)

Treatment Group and Animal No.		7				8				9			
		Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd
Y84		2.0	85	46	1560	1.1	33	48	2147	1.1	8	36	500
Y50		.4	0	59	1562	.7	0	48	364	.7	0	50	350
A ⁺ + Testosterone													
Y80		.6	80	-f	0	.3	0	-f	0	1.0	0	-f	0
B19		1.5	50	14	1361	1.6	43	4	1395	1.6	80	26	1292
Y100		.6	0	-f	0	.6	0	-f	0	.5	0	-f	0
G87		.9	70	5	0	1.0	72	32	643	.4	15	41	315
Y46		.4	0	-f	0	.3	0	-f	0	.3	0	-f	0
Control Irradiated													
G90		1.1	10	77	1663	1.3	8	74	1834	.4	5	82	449
Y79		.8	70	70	1008	.5	13	68	1166	.3	10	54	108
A ⁻ Irradiated													
G92		.8	10	84	1370	.8	73	30	897	.5	43	18	456

TABLE 24 (Continued)

Treatment Group and Animal No.	10			11			12		
	Vol. a	Mot. b	Abn. c TCPd	Vol. a	Mot. b	Abn. c TCPd	Vol. a	Mot. b	Abn. c TCPd
Control									
13	2.1	93	47 5296	2.0	90	0 352	1.2	90	0 877
15	1.0	0	52 324	2.1	0	9 231	.8	0	f 0
Y97	.4	50	3 20	1.2	85	33 938	.5	0	f 0
22	.8	48	3 288	.8	85	3 268	1.9	90	37 2825
G9	.7	75	14 385	.5	75	7 168	1.0	70	59 330
Y43	2.0	95	35 3840	.9	80	31 1224	2.5	95	4 8820
Deficient									
Y19	.8	3	57 290	2.0	0	23 470	.9	90	36 900
B9	.9	75	17 984	.7	13	48 444	- ^e	-	-
Y44	.5	0	33 8	.4	0	50 12	15	0	77 450
Returned									
G99	1.6	88	28 2942	1.2	80	12 1476	1.9	95	5 1771
G94	1.1	83	3 1902	.7	80	7 196	1.3	85	0 514
Y47	.9	70	1 764	.8	88	5 819	1.1	90	30 2606

TABLE 24 (Continued)

Treatment Group and Animal No.	10				11				12			
	Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd
B2	.6	80	21	1664	1.2	93	7	2627	1.2	50	8	288
Y22	.6	88	15	717	1.3	80	33	884	1.0	95	66	2066
Y99	.6	50	9	203	1.4	90	23	1870	1.4	80	7	854
A ⁻ + Thyroprotein												
Y76	1.5	48	32	777	3.6	95	36	5987	2.5	90	61	4788
Y24	.7	80	55	1400	.6	85	47	906	.9	90	28	1292
Y83	.5	0	f	0	.4	0	f	0	.6	0	f	0
Y98	.4	15	15	43	.4	15	38	48	.7	60	35	953
G26	1.2	60	15	2968	.4	40	39	957	.6	40	f	0
Y75	1.5	5	42	653	6.0	0	71	1260	4.0	0	32	1000
A ⁻ + PNS												
B21	.2	0	f	0	.1	0	f	0	.2	0	f	0
Y81	.7	30	25	21	.7	40	34	152	.5	30	29	310
Y20	.5	0	f	0	.7	3	f	0	.5	5	f	0
Y84	.2	10	80	150	e	-	-	-	-	-	-	-
Y50	.4	0	65	237	.4	0	50	14	.3	0	100	105

TABLE 24 (Continued)

Treatment Group and Animal No.	10			11			12		
	Vol. ^a	Mot. ^b	Abn. ^c TCP ^d	Vol. ^a	Mot. ^b	Abn. ^c TCP ^d	Vol. ^a	Mot. ^b	Abn. ^c TCP ^d
A⁻ + Testosterone									
Y80	.8	5	f -	.5	0	f -	.5	0	f -
B19	.5	65	9	.9	50	38	3.5	5	62
Y400	.3	0	f -	.3	0	f -	- ^e	-	-
G87	.3	0	31	.4	0	25	- ^e	-	-
Y46	.4	0	f -	.2	0	f -	.4	0	f -
Control Irradiated									
G90	.8	0	80	.6	5	50	.7	20	47
Y79	.7	10	42	.5	60	40	.3	15	60
A⁻ Irradiated									
G92	.7	85	20	.5	30	50	.3	85	48
			255			255			363

TABLE 24 (Cont Inued)

Treatment Group and Animal No.	13				14				15			
	Vol. a	Mot. b	Abn. c	TCP d	Vol. a	Mot. b	Abn. c	TCP d	Vol. a	Mot. b	Abn. c	TCP d
Control												
13	.6	90	34	983	1.3	90	8	1638	1.1	90	4	275
15	1.2	5	33	348	1.6	30	6	800	.4	85	4	464
Y97	1.2	90	0	1361	.4	95	18	1774	3.0	90	0	840
22	.5	95	30	1021	1.1	90	11	1774	1.1	90	14	1760
G9	.7	20	9	500	2.1	90	37	2699	.5	20	40	50
Y43	3.0	90	9	4989	2.4	80	31	4114	2.4	80	31	4114
Deficient												
Y19	1.2	80	12	792	.7	70	18	350	.4	15	62	106
Y44	.7	0	-f	0	-e	-	-	-	-	-	-	---
Returned												
G99	4.0	80	4	2260	.6	20	0	9	1.9	90	6	1723
G94	1.0	20	70	550	.6	90	14	559	.3	80	11	29
Y47	1.3	90	5	910	1.1	95	33	2717	.5	70	8	123

TABLE 24 (Continued)

Treatment Group and Animal No.	13				14				15			
	Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd
B2	.4	80	7	138	1.0	95	13	1789	1.3	80	0	1375
Y22	1.2	85	28	1966	1.5	80	20	2420	.7	95	16	416
Y99	1.0	80	0	650	1.2	40	31	1874	1.1	95	15	1497
A ⁺ + Thyroprotein												
Y76	2.0	80	29	3276	.5	40	30	795	2.1	85	11	3704
Y24	2.0	50	46	2528	.8	90	24	1794	.4	90	13	766
Y83	.5	5	67	8	.5	80	0	807	1.0	0	76	125
Y98	1.2	85	4	324	.3	70	0	21	.6	80	6	290
G26	.7	10	55	1129	.6	20	64	846	.9	5	68	702
Y75	1.2	0	- ^f	0	2.0	0	100	600	3.0	0	100	315
A ⁺ + PMS												
Y81	.2	5	35	17	.4	5	47	72	.3	0	22	27
Y20	.3	20	36	74	- ^e	-	-	-	-	-	-	-
Y50	.2	5	2	62	.5	0	- ^f	0	.7	0	100	11

TABLE 24 (Continued)

Treatment Group and Animal No.	13			14			15		
	Vol. ^a	Mot. ^b	Abn. ^c TCPd	Vol. ^a	Mot. ^b	Abn. ^c TCPd	Vol. ^a	Mot. ^b	Abn. ^c TCPd
A⁻ + Testosterone									
Y80	.7	90	- ^f 0	.8	5	62	.3	0	- ^f 0
B19	1.2	0	- ^f 0	.5	0	- ^f 0	.4	0	- ^f 0
Y46	.4	0	- ^f 0	.4	0	- ^f 0	.2	0	- ^f 0
Control Irradiated									
G90	1.0	85	24	.8	90	19	.1	40	15
Y79	.7	70	45	.7	90	82	.2	20	65
A⁻ Irradiated									
G92	.6	50	13	1.0	70	19	.1	10	15
----- 16 ----- 17 ----- 18 -----									
Control									
13	1.1	60	37	1.0	90	15	2.9	95	8
15	.5	90	5	6.0	5	5	2.8	60	7
Y97	3.0	95	14	2.0	95	13	1.6	90	16
22	1.6	90	35	1.0	95	66	1.1	90	17
									583

TABLE 24 (Continued)

Treatment Group and Animal No.	16			17			18					
	Vol. a	Mot. b	Abn. c	TCpd	Vol. a	Mot. b	Abn. c	TCpd	Vol. a	Mot. b	Abn. c	TCpd
G9	1.3	60	36	1901	1.0	90	43	1915	2.5	85	47	5230
Y43	.8	85	8	912	.8	30	18	196	1.0	90	16	3856
Deficient												
Y19	1.1	15	75	1885	1.4	5	81	728	.5	15	78	1437
Returned												
G99	.5	0	- ^f	0	1.0	65	3	165	1.0	90	9	1220
G94	.3	25	7	384	1.0	70	0	310	1.3	80	0	767
Y47	.9	90	19	1836	1.7	95	17	3941	1.0	70	11	720
B2	2.6	95	19	3539	.9	80	0	14	.9	70	3	522
Y22	.6	70	15	408	.5	20	13	60	1.1	70	44	2079
Y99	1.1	90	12	1525	1.8	90	55	792	1.0	90	13	1336
A ⁺ + Thyroprotein												
Y76	1.2	85	29	3145	1.4	95	16	1729	1.1	95	41	804
Y24	.7	95	27	1429	.9	80	34	1270	.5	903	30	378
Y83	.4	15	12	276	.4	5	33	424	1.0	5	20	200
Y98	.6	30	23	420	.3	30	51	575	.4	15	32	1350

TABLE 24 (Continued)

Treatment Group and Animal No.	16			17			18					
	Vol. ^a	M.c.t. ^b	Abn. ^c TcPd	Vol. ^a	Mot. ^b	Abn. ^c TcPd	Vol. ^a	M.c.t. ^b	Abn. ^c TcPd			
G26	.e	-	-	-	-	-	-	-	-			
Y75	.e	-	-	-	-	-	-	-	-			
A ⁺ + PMS												
Y81	.3	0	-f	0	.3	0	-f	0	.3	0	67	5
Y50	.2	0	-f	0	.4	0	-	0	.6	0	-f	0
A ⁺ + Testosterone												
Y80	.4	0	-f	0	.5	0	-f	0	.3	0	-f	0
B19	1.9	0	-f	0	3.0	0	-f	0	2.2	0	-f	0
Y46	.2	0	-f	0 0	.3	0	-f	0	.e	-	-	-
Control Irradiated												
G90	.6	15	61	1285	.4	40	13	30	1.3	15	92	3860
Y79	.5	80	22	520	.7	30	18	480	1.0	90	7	2520
A ⁺ Irradiated												
G92	.5	5	45	5	.3	50	57	578	1.4	10	57	518

TABLE 24 (Continued)

Treatment Group and Animal No.	19			20			21		
	Vol. a	Mot. b	Abn. c	Vol. a	Mot. b	Abn. c	Vol. a	Mot. b	Abn. c
	TCPd			TCPd			TCPd		
Control									
13	.5	50	46	327	.5	85	0	475	1.1 85 17 1441
15	2.1	5	9	2858	.5	80	2	1361	1.5 85 3 3440
Y97	3.2	80	2	3549	1.2	90	6	408	1.9 50 2 399
22	1.3	85	24	1343	1.7	95	19	2013	1.2 80 6 1114
G9	1.0	5	31	1714	2.0	95	14	3226	1.5 80 32 1890
Y43	2.5	5	31	5293	3.9	20	50	9828	.8 20 18 1209
Deficient									
Y19	.9	85	44	1499	.5	5	79	595	.7 5 89 161
Returned									
G99	.9	95	26	2313	4.0	95	4	9272	2.2 90 0 2827
G94	.8	10	0	324	3.3	95	41	4076	.7 80 4 651
Y47	1.3	40	14	1606	1.0	85	24	1663	1.5 50 0 53
B2	1.8	90	37	3220	2.5	95	7	2583	1.3 90 15 715
Y22	1.0	40	28	285	2.5	70	17	3655	1.5 80 32 890
Y99	.7	10	0	159	.5	0	-f	0	.5 10 14 108

TABLE 24 (Continued)

Treatment Group and Animal No.	19				20				21			
	Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd	Vol. a	Mot. b	Abn. c	TCPd
A ⁻ + Thyroprotein												
Y76	.8	95	38	1753	1.8	95	56	3992	2.0	90	19	1500
Y24	.6	0	55	575	.6	5	88	650	.7	0	76	370
Y83	1.1	0	-f	0	.3	0	-f	0	.6	0	-f	0
Y98	1.0	10	89	2671	.7	10	76	1481	-.e	-	-	--
A ⁻ + PMS												
Y50	.6	0	-f	0	.3	0	-f	20	.5	0	-f	0
A ⁻ + Testosterone												
Y80	.2	0	-f	0	.3	0	-f	0	.1	0	-f	0
B91	-.e	-	-	-	-	-	-	-	-	-	-	-
Control Irradiated												
G90	1.4	40	68	3951	.3	10	69	162	1.4	40	82	3458
Y79	.5	10	19	1122	1.0	80	56	2293	1.0	90	29	1991
A ⁻ Irradiated												
G92	.8	20	51	536	1.0	50	36	330	.2	5	58	156

aVolume of semen in milliliters

cPer cent abnormal sperm cells

bEstimated per cent motile sperm cells

dTotal number of sperm cells per ejaculate times 10⁶

eAnimal died

fConcentration too low for a differential count

TABLE 25

SUMMARY OF INDIVIDUAL RAM ENDOCRINE GLAND WEIGHT IN GRAMS
PER 100 LBS. BODY WEIGHT

Treatment Group and Animal No.	Pituitary	Adrenals	Thyroid	Cowpers	Seminal Vesicles	Epididymis	Testes
Control							
13	.635	1.7	2.5	1.9	9.9	42.5	311.8
15	.670	2.1	3.2	2.9	13.7	39.9	269.4
Y97	.738	1.8	2.4	2.4	14.9	44.5	280.4
22	.512	1.5	1.9	1.9	14.4	14.4	336.9
G9	.675	2.2	3.9	2.4	10.1	61.7	302.0
Y43	.575	3.7	7.7	1.7	10.1	41.1	182.3
Av.	.634	2.2	3.6	2.4	36.6	40.7	280.5
Deficient							
Y19	1.073	3.4	2.5	1.3	9.7	35.3	105.4
B9	^a	3.2	4.1	1.4	6.8	31.3	101.2
B32	.565	2.2	2.9	3.4	13.1	57.4	174.6
B83	.551	3.1	2.7	3.5	7.6	46.0	116.4
Y44	.427	4.0	4.3	2.9	24.8	63.9	88.9
Av.	.709	3.2	3.2	2.3	35.8	44.9	115.7

TABLE 25 (Continued)

Treatment Group and Animal No.	Pituitary	Adrenals	Thyroid	Cowpers	Seminal Vesicles	Epididymis	Testes
Returned							
G99	.582	1.8	2.8	2.5	12.0	45.9	274.5
G94	1.008	2.1	1.8	2.8	15.0	40.2	235
Y47	.491	2.1	5.0	2.9	8.7	50.5	205.7
B2	.628	1.9	2.0	2.4	21.8	48.6	268.6
Y22	.500	2.0	3.4	2.6	12.4	59.2	312.1
Y99	.548	2.0	8.2	1.2	6.9	44.9	214.7
Av.	.626	2.0	3.9	2.8	38.4	48.3	268.5
A ⁺ + Thyroprotein							
Y76	.457	2.2	2.8	1.5	11.6	49.3	379.1
Y24	.459	2.9	3.5	1.5	8.9	32.1	81.3
Y83	.247	3.3	3.9	1.6	6.0	22.7	87.4
Y98	.383	3.9	3.2	2.5	11.7	42.2	116.8
G26	— ^a	— ^a	— ^a	— ^a	— ^a	— ^a	— ^a
Y75	.520	2.8	3.9	4.1	9.9	26.1	97.8
Av.	.342	2.8	3.6	2.9	29.1	33.1	143.4

TABLE 25 (Continued)

Treatment Group and Animal No.	Pituitary	Adrenals	Thyroid	Cowpers	Seminal		
					Vesicles	Epididymis	Testes
A ⁻ + PMS							
B21	.682	a	7.9	2.9	20.2	49.0	82.5
3	.780	2.8	3.0	1.3	4.0	36.1	141.8
Y81	.545	2.5	3.4	2.0	12.3	25.3	74.3
Y20	.304	3.3	3.5	2.8	7.2	29.3	88.9
Y84	.444	4.0	3.7	4.6	11.2	61.8	109.7
Y50	.606	3.8	5.1	2.2	7.6	29.7	129.4
Av.	.560	4.5	3.2	2.6	31.3	38.6	104.5
A ⁻ + Testosterone							
Y80	.654	6.5	2.5	5.1	98	30.3	91.6
B19	.377	6.4	3.1	3.1	10.2	33.7	96.5
Y100	.612	6.1	3.9	a	16.8	46.1	99.8
Y95	.411	4.0	6.9	7.0	7.4	22	61.4
G87	.603	3.7	4.7	3.7	5.7	18.5	40.5
Y46	1.404	6.2	4.1	2.9	14.9	53.4	100.0
Av.	.684	5.5	4.2	4.8	32.5	35.0	81.7

TABLE 25 (Continued)

Treatment Group and Animal No.	Pituitary	Adrenals	Thyroid	Cowpers	Seminal Vesicles	Epididymis	Testes
Control Irradiated							
G90	.596	1.9	3.0	2.4	17.2	57.6	373.0
G97	.493	3.3	3.6	1.3	9.9	37.3	134.0
6	.671	- ^a	2.7	1.3	8.8	42.7	187.3
21	.428	2.7	2.7	1.7	10.9	52.0	200.1
Y79	.540	2.3	2.9	1.5	7.4	33.4	208.9
16	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a
Av.	.545	2.5	3.0	1.6	30.8	42.8	219.0
A ⁻ Irradiated							
G1	.761	4.5	5.9	1.1	4.7	39.3	123.1
G92	.718	2.4	3	1.4	6.8	31.1	102.4
Y74	.895	4.3	6.6	2.1	14.2	43.0	189.2
Y85	.356	4.9	9.1	2.0	5.2	61.1	227.9
B52	.382	4.8	5.9	1.5	6.5	55.4	239.4
37	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a
Av.	.583	4.3	6.6	1.6	22.0	47.6	186.9

^a Sample lost at biopsy.

TABLE 26

ANALYSIS OF VARIANCE FOR THE DIFFERENCE IN AVERAGE
DAILY GAIN DURING PHASE I

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Group	2	.047	.024
Subgroup	7	.262	.037*
Error	37	.433	.012
Total	46	.742	

* $P < .05$

TABLE 27

ANALYSIS OF VARIANCE FOR THE DIFFERENCE IN AVERAGE
DAILY GAIN DURING PHASE II

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments	7	.748	.107*
Groups	2	.441	.220*
Treat. x Group	12	.249	.027
Error	24	.490	.020
Total	45	2.028	

* $P < .01$

TABLE 28

ANALYSIS OF VARIANCE FOR THE DIFFERENCE IN AVERAGE
DAILY GAIN DURING PHASES I AND II COMBINED

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments	7	.177	.025**
Groups	2	.003	.002
Treat. x Groups	14	.227	.016*
Error	23	.102	.004
Total	46	.509	

* $P < .05$

** $P < .01$

TABLE 29

ANALYSIS OF VARIANCE FOR THE DIFFERENCE IN SURVIVAL
TIME

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments	7	56762	8108.9*
Groups	2	7993	3996.5
Treat. x Group	14	20181	1441.5
Error	24	32475	1353.1
Total	47	117411	

* $P < .01$

TABLE 30

ANALYSIS OF VARIANCE MEAN SQUARES FOR SEMEN VOLUME, MOTILITY,
ABNORMAL CELLS AND TOTAL SPERM CELL PRODUCTION--WEEK 1

Source of Variance	Degrees of Freedom	Mean Squares		
		Semen Volume	Semen Motility	Total Abnormal cells Total Sperm Production
Treatment	7	10.46	815.59	288.90
Group	2	2.24	425.27	2489.91
Treatment x Group	14	5.47	397.8	363.96
Error	23	3.61	475.35	512.89
				2694.20
				906.58
				1882.12
				1910.85

TABLE 31

ANALYSIS OF VARIANCE MEAN SQUARES FOR SEMEN VOLUME, MOTILITY,
ABNORMAL CELLS AND TOTAL SPERM CELL PRODUCTION--WEEK 2

Source of Variance	Degrees of Freedom	Mean Squares			
		Semen Volume	Semen Motility	Total Abnormal Cells	Total Sperm Production
Treatment	7	16.86	1323.85	986.74	6197.70
Group	2	18.60	926.55	5838.28	1099.78
Treatment x Group	14	11.53	352.38	644.08	352.38
Error	23	9.09	608.59	1399.11	5839.75

TABLE 32

ANALYSIS OF VARIANCE MEAN SQUARES FOR SEMEN VOLUME, MOTILITY,
ABNORMAL CELLS AND TOTAL SPERM CELL PRODUCTION--WEEK 3

Source of Variance	Degrees of Freedom	Mean Squares			
		Semen Volume	Semen motility	Total Abnormal Cells	Total Sperm Production
Treatment	7	34.62	830.56	3299.84	14722.34
Group	2	3.51	1613.42	43706.72	59304.49
Treatment x Group	14	32.13	793.98	4164.50	16134.25
Error	22	35.32	1064.43	4524.52	11992.54

TABLE 33

Source of Variance	Degrees of Freedom	Semen Volume	Semen Motility	Mean Squares	
				Total Abnormal Cells	Total Sperm Production
Treatment	7	72.80	1697.59	9245.48	9245.48
Group	2	73.51	2360.75	23486.80	23486.80
Treatment $\times$ Group	14	30.56	645.20	7480.30	7473.14
Error	22	37.20	916.84	9798.30	9798.30

TABLE 34

ANALYSIS OF VARIANCE MEAN SQUARES FOR SEMEN VOLUME, MOTILITY,
ABNORMAL CELLS AND TOTAL SPERM CELL PRODUCTION--WEEK 5

Source of Variance	Degrees of Freedom	Mean Squares			
		Semen Volume	Sperm Motility	Total Abnormal Cells	Total Sperm Production
Treatment	7	55.34	2188.60	9579.57	25469.07
Group	2	182.32	4313.69	22239.61	63242.62
Treatment x Group	14	17.77*	909.60	2587.46**	8340.22*
Error	19	49.42	1098.68	7527.60	26832.45

* $p < .05$

TABLE 35
ANALYSIS OF VARIANCE MEAN SQUARES FOR SEMEN VOLUME, MOTILITY,
ABNORMAL CELLS AND TOTAL SPERM CELL PRODUCTION--WEEK 6

Source of Variance	Degrees of Freedom	Mean Squares			
		Semen Volume	Semen Motility	Total Abnormal Cells	Total Sperm Production
Treatment	7	23.50	1791.13	11460.54 [*]	24965.27
Group	2	57.99	523.09	12544.60	63456.64
Treatment x Group	12	31.29	1353.55	2347.16	12317.67
Error	15	21.80	1170.83	2728.10	9222.67

*p < .01

TABLE 36

ANALYSIS OF VARIANCE MEAN SQUARES FOR SEMEN VOLUME, MOTILITY,
ABNORMAL CELLS AND TOTAL SPERM CELL PRODUCTION--WEEK 7

Source of Variance	Degrees of Freedom	Mean Squares			
		Semen Volume	Semen Motility	Total Abnormal Cells	Total Sperm Production
Treatment	7	16.49	2030.21	3081.91 [*]	10120.71
Group	2	3.23	574.16	3047.60	18245.97
Treatment x Group	11	20.02	1210.57	1460.36 [*]	10892.66
Error	14	22.32	1133.03	322.21	6070.43

^{*}P < .01

TABLE 37

ANALYSIS OF VARIANCE MEAN SQUARES FOR SEMEN VOLUME, MOTILITY,
ABNORMAL CELLS AND TOTAL SPERM CELL PRODUCTION--WEEK 8

Source of Variance	Degrees of Freedom	Mean Squares			
		Semen Volume	Semen Motility	Total Abnormal Cells	Total Sperm Production
Treatment	7	20.62	2667.67	2243.03*	14568.89
Group	2	7.73	1189.72	5908.25	60919.81
Treatment x Group	11	22.32	582.35	737.59	8823.65
Error	13	42.23	971.96	480.31	5829.23

* $p < .01$

TABLE 38

ANALYSIS OF VARIANCE MEAN SQUARES FOR SEMEN VOLUME, MOTILITY,
ABNORMAL CELLS AND TOTAL SPERM CELL PRODUCTION--WEEK 9

Source of Variance	Degrees of Freedom	Mean Squares			
		Semen Volume	Semen Motility	Total Abnormal Cells	Total Sperm Production
Treatment	7	12.11	4175.50*	224.16	6095.45
Group	2	10.92	614.44	1157.85	13696.41
Treatment x Group	11	15.65	330.46	255.24	5244.51
Error	13	9.19	1264.77	353.15	4251.19

* $p < .05$

TABLE 39
ANALYSIS OF VARIANCE MEAN SQUARES FOR SEMEN VOLUME, MOTILITY,
ABNORMAL CELLS AND TOTAL SPERM CELL PRODUCTION--WEEK 10

Source of Variance	Degrees of Freedom	Mean Squares			
		Semen Volume	Semen Motility	Total Abnormal Cells	Total Sperm Production
Treatment	7	35.0	3411.86*	2459.56	19544.98
Group	2	45.13	324.41	4221.60	19620.41
Treatment x Group	11	17.58	928.41	1186.28	9807.43
Error	13	16.61	749.85	3107.0	17350.58

*p < .01

TABLE 40

ANALYSIS OF VARIANCE FOR THE DIFFERENCE IN MEAN SIZE
OF THE TESTES

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments	7	124065	13.6*
Groups	2	4920	1.9
Error	14	18197	
Total	23	147182	

* $P < .01$

TABLE 41

ANALYSIS OF VARIANCE FOR THE DIFFERENCE IN MEAN SIZE
OF THE EPIDYDIMIS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments	7	652.0	93.1*
Groups	2	992.8	496.4*
Error	14	156.8	11.2
Total	23	1801.6	

* $P < .01$

TABLE 42

ANALYSIS OF VARIANCE FOR THE DIFFERENCE IN MEAN SIZE
OF THE ADRENAL GLANDS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments	7	29.1	4.15*
Groups	2	.5	.25
Error	14	5.2	.37
Total	23	34.8	

* $P < .01$

TABLE 43

ANALYSIS OF VARIANCE FOR THE DIFFERENCE IN MEAN SIZE
OF THE SEMINAL VESICLES

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments	7	64.0	9.1
Groups	2	7.4	3.7
Error	14	81.6	5.83
Total	23	153.0	

TABLE 44

ANALYSIS OF VARIANCE FOR THE DIFFERENCE IN MEAN SIZE
OF THE COWPER'S GLANDS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatment	7	21.8	3.11*
Group	2	2.8	1.4
Error	14	11.1	.79
Total	23	35.7	

* $P < .05$

TABLE 45

ANALYSIS OF VARIANCE FOR THE DIFFERENCE IN MEAN SIZE
OF THE THYROID

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments	7	26.7	3.81*
Group	2	4.2	2.1
Error	14	18.6	1.33
Total	23	49.5	

* $P < .05$

TABLE 46
ANALYSIS OF VARIANCE FOR THE DIFFERENCE IN MEAN SIZE
OF THE PITUITARY GLAND

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatment	7	.42	.06*
Group	2	.28	.14*
Error	14	.134	.01
Total	23	.834	

* $P < .01$

BIOGRAPHICAL SKETCH

Enrique Sosa was born March 17, 1940 at Camaguey, Cuba. In June 1957 he was graduated from Colegio de Belen in Habana, Cuba. He received the degree of Bachelor of Science in Agriculture in January 1961 at the University of Florida. He enrolled in the Graduate School of the University of Florida in February 1961 and obtained a degree of Master of Science in Agriculture in June 1962.

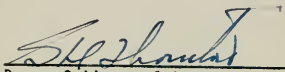
Membership is maintained in the American Society of Animal Science, Phi Sigma and Sigma Xi.

He is married to the former Irene Tremols and is the father of two daughters.

The author is now a candidate for the degree Doctor of Philosophy.

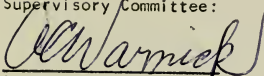
This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

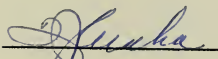
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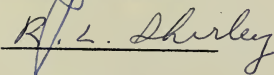

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Dean, Graduate School

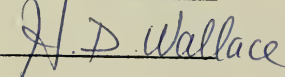
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